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# Distinguishing Seven Species of *Bacillus* Spores Using BioAerosol Mass Spectrometry

D. P. Fergenson, M. E. Pitesky, M. Frank, J. M. Horn, E. E. Gard

October 18, 2005

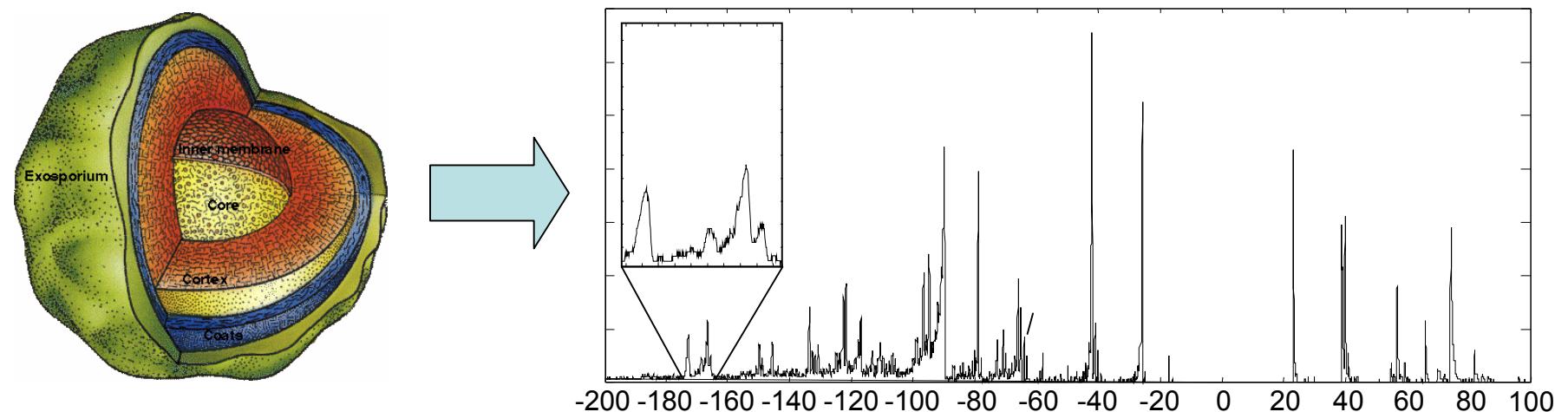
The American Association for Aerosol Research Annual Meeting  
Austin, TX, United States  
October 17, 2005 through October 21, 2005

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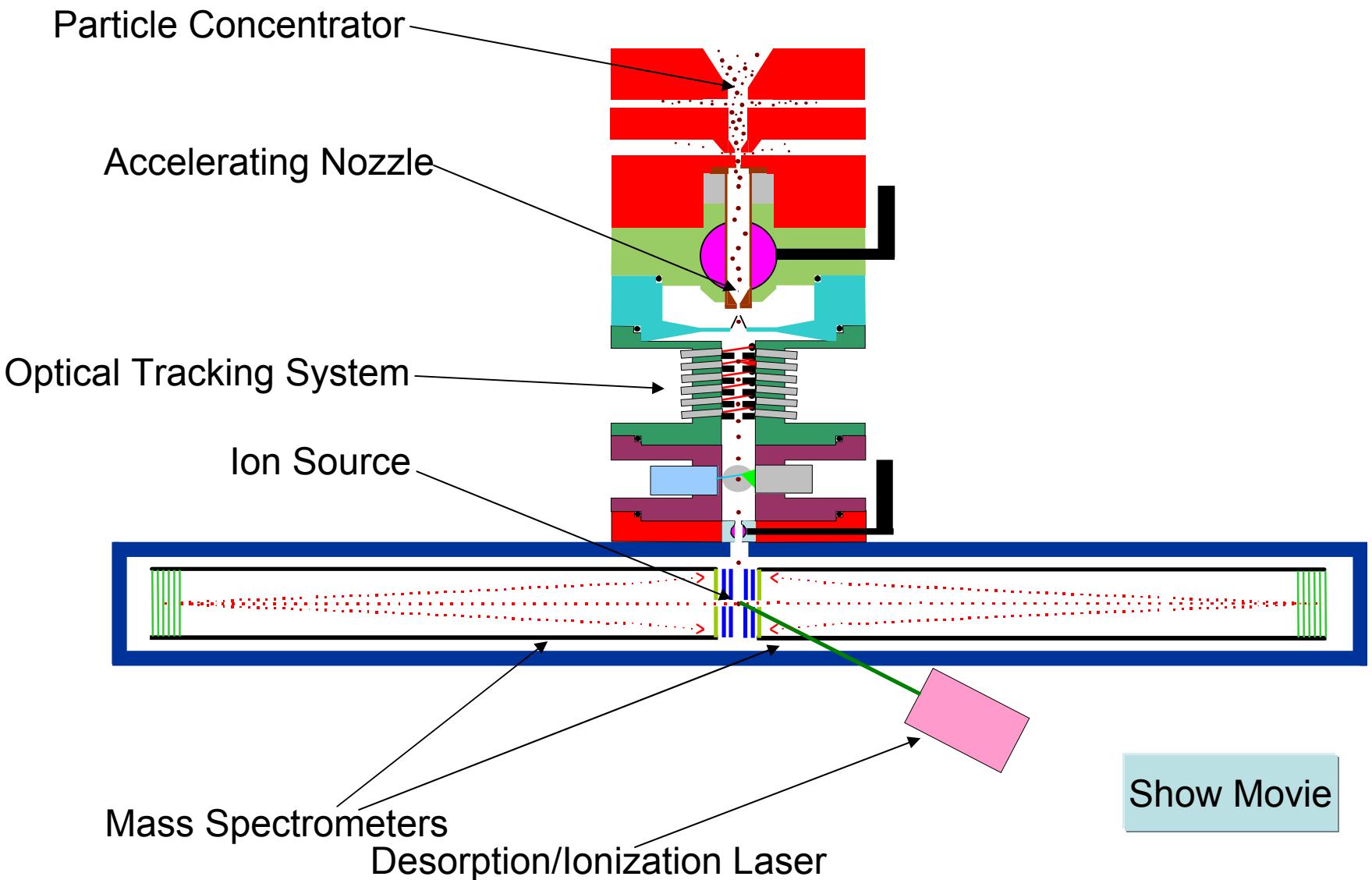
# Distinguishing Seven Species of Bacillus Spores Using BioAerosol Mass Spectrometry



*David P. Fergenson, Maurice E. Pitesky, Matthias Frank, Joanne M. Horn and Eric E. Gard*  
*Lawrence Livermore National Lab*  
*before the AAAR, October 18, 2005*



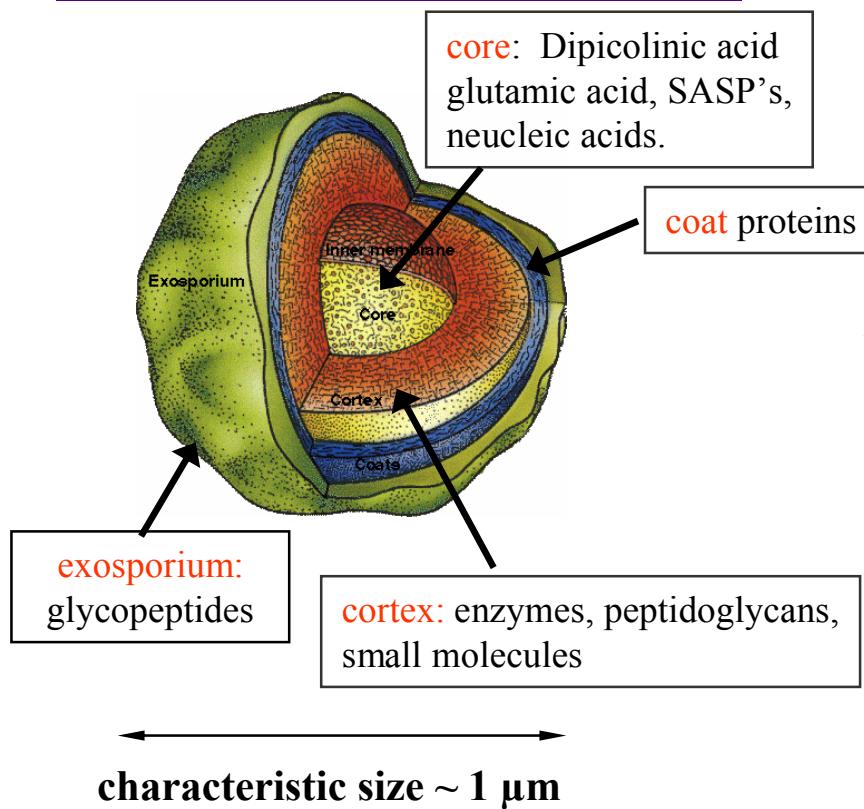
# BioAerosol Mass Spectrometry (BAMS)



# Anatomy of a Bacterial Spore

- Dormant form of bacteria
- Formation induced by environmental stress

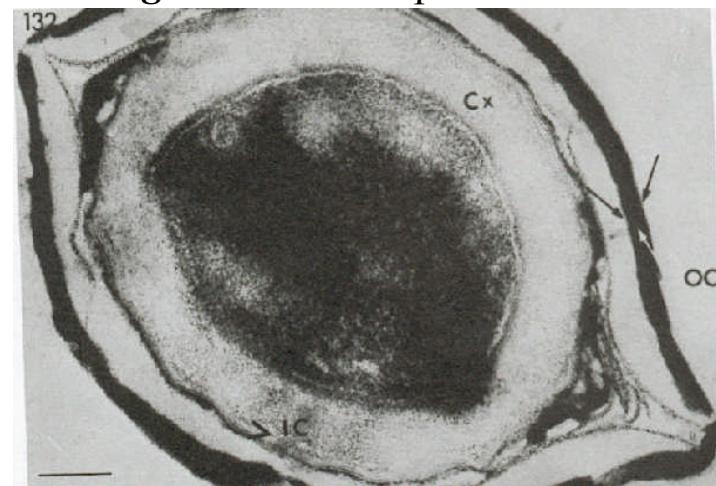
Diagram of a bacterial spore



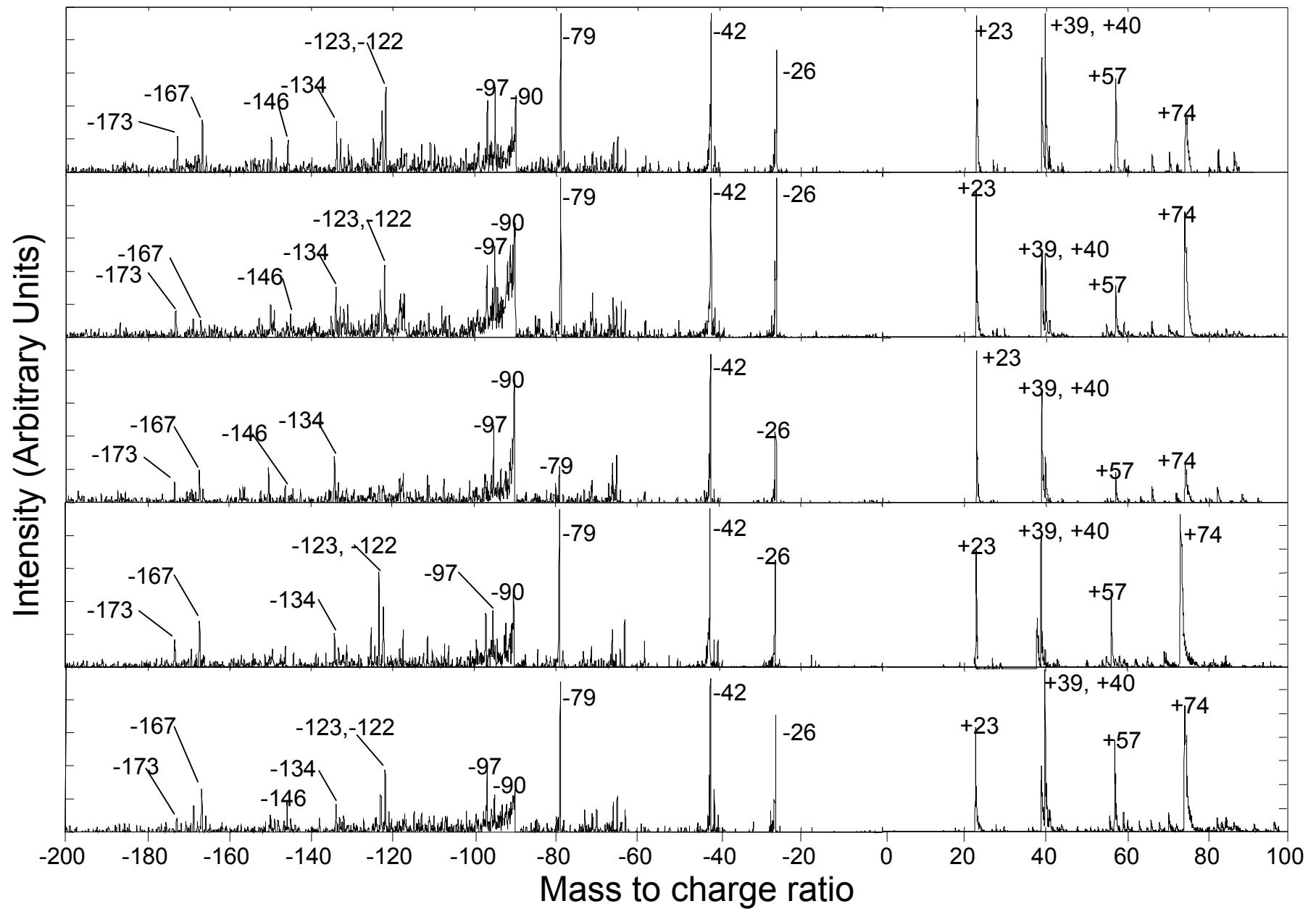
*Bacillus licheniformis* endospore exterior



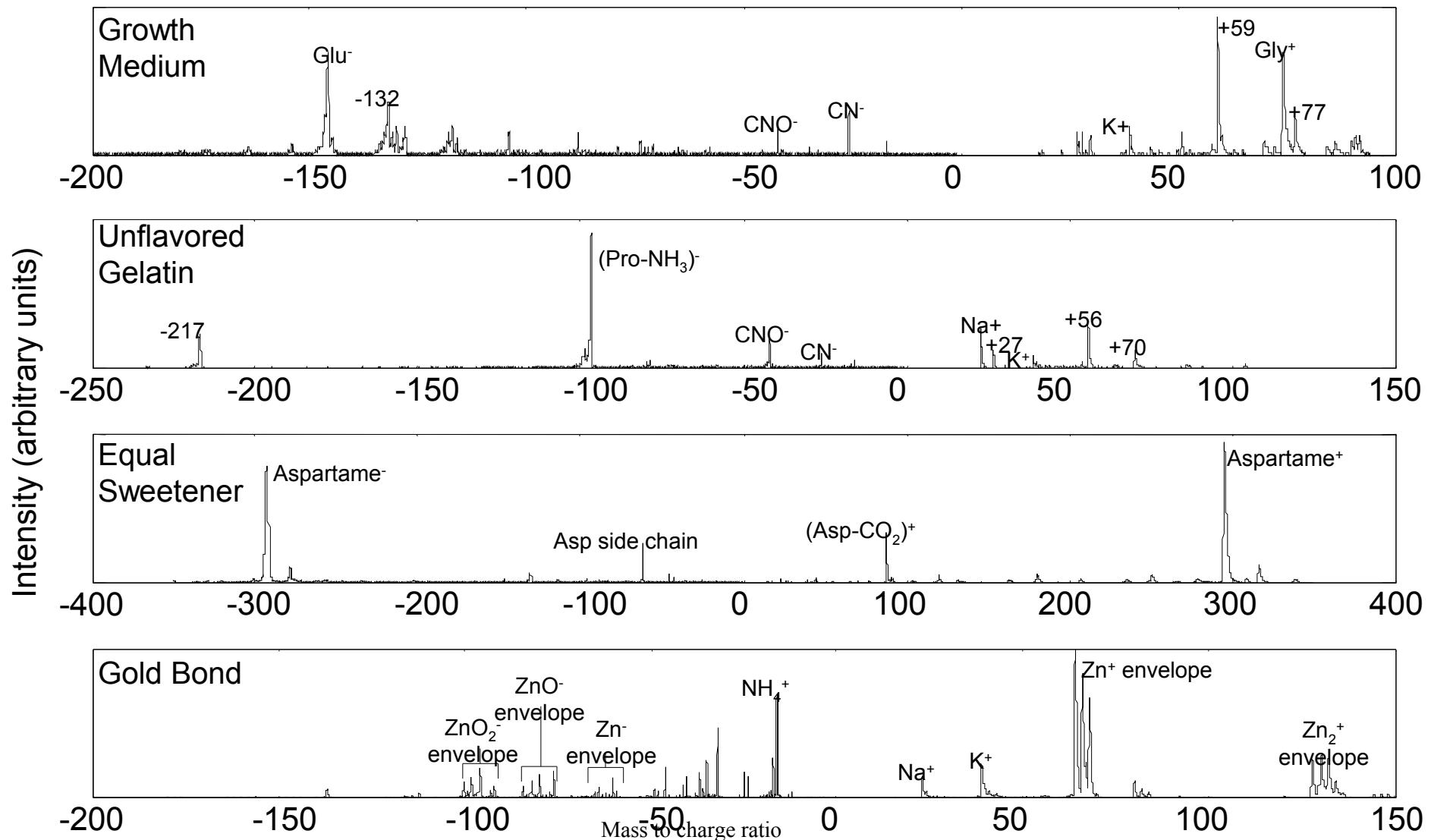
*Bacillus megaterium* endospore cross section



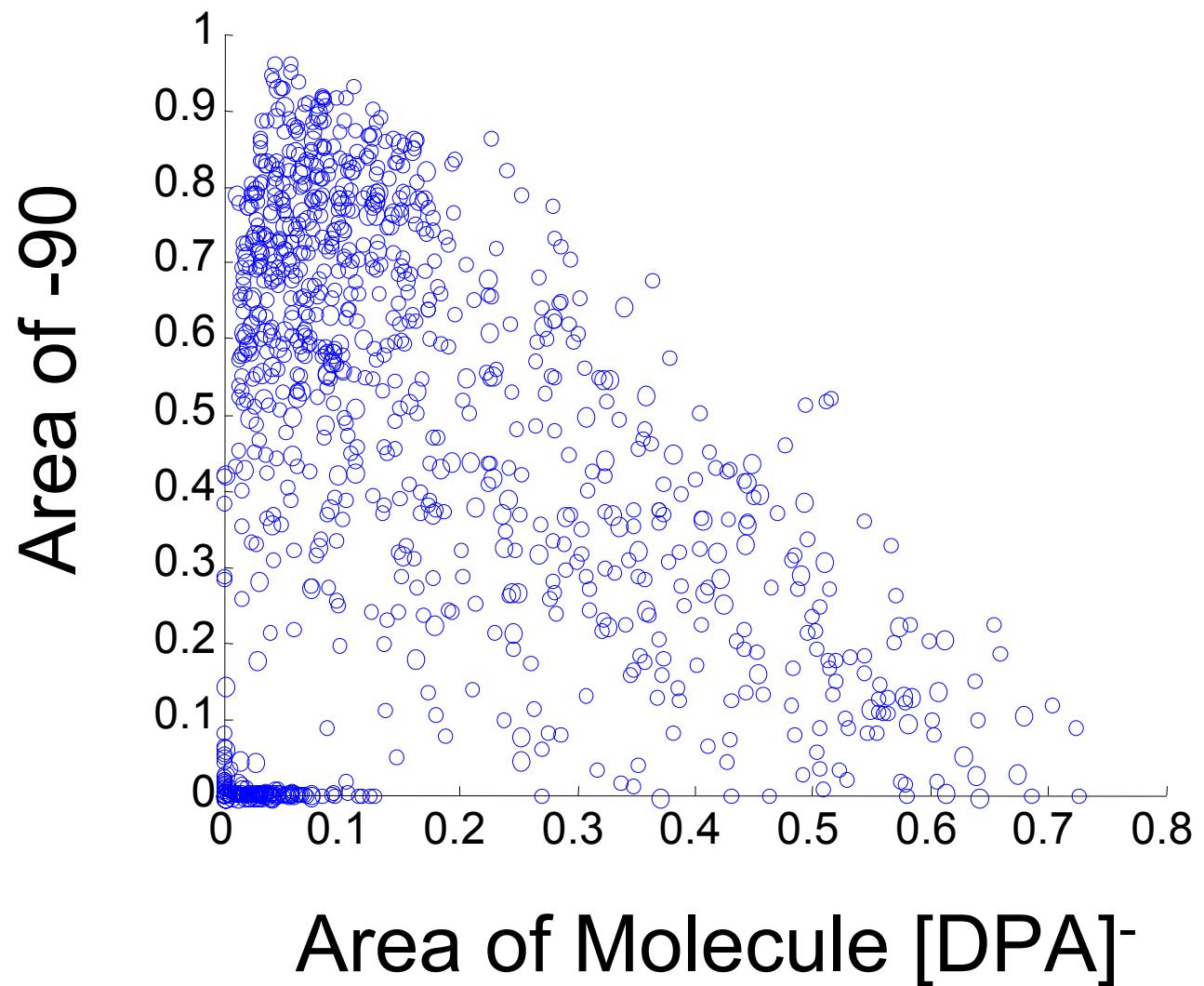
# Bacillus Spore Data Variability



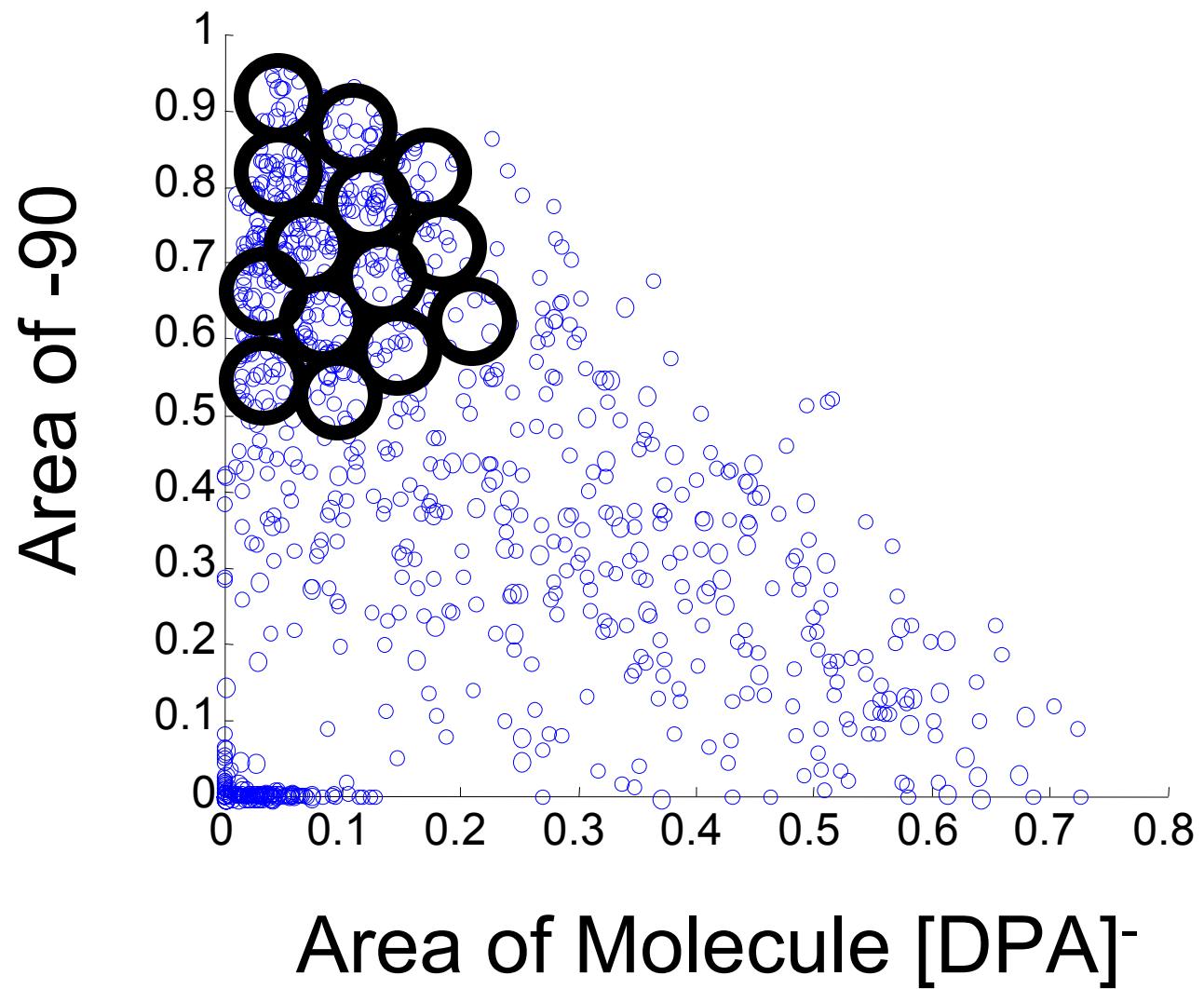
# Suspicious White Powders



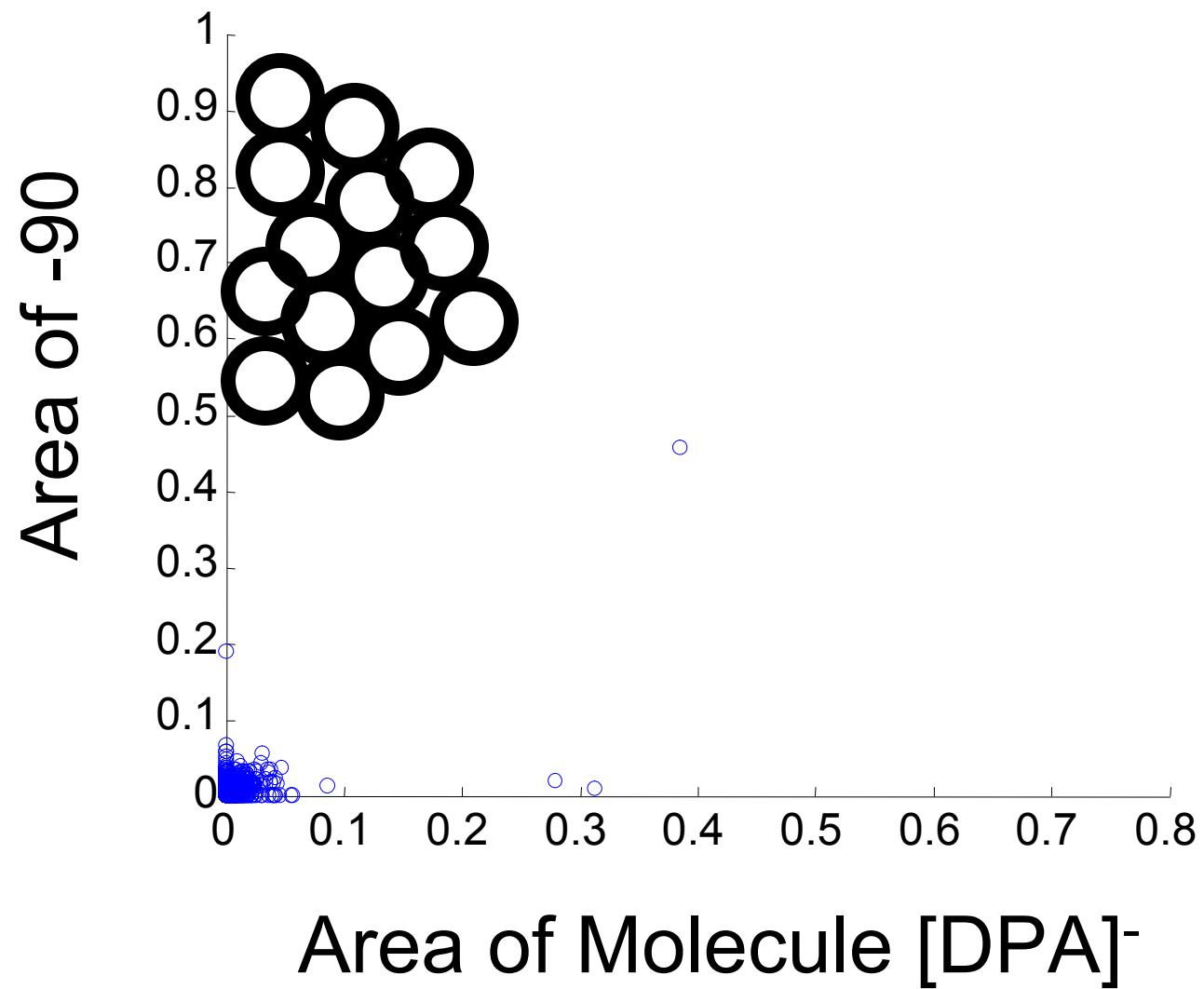
Amount of -90 (adenine fragment) vs DPA<sup>-</sup>,  
1000 Spectra of *B. cereus* Spores



Amount of -90 (adenine fragment) vs DPA<sup>-</sup>,  
1000 Spectra of *B. cereus* Spores



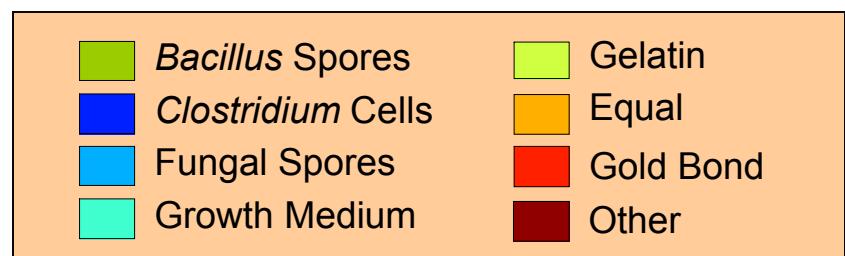
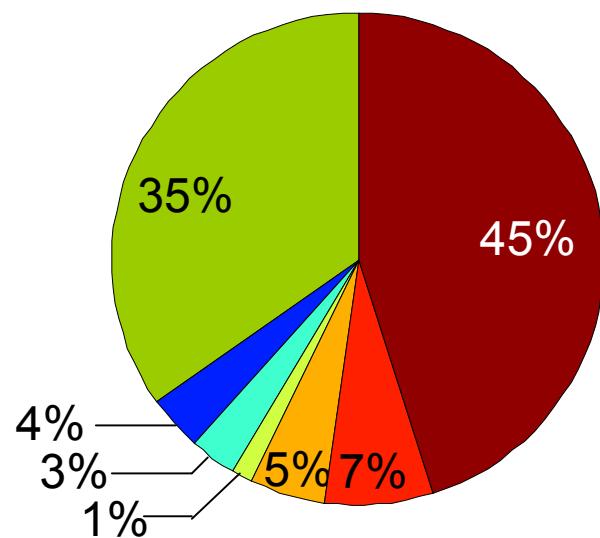
Amount of -90 (adenine fragment) vs DPA<sup>-</sup>,  
1000 Spectra of *C. acetobutylicum* Vegetative Cells



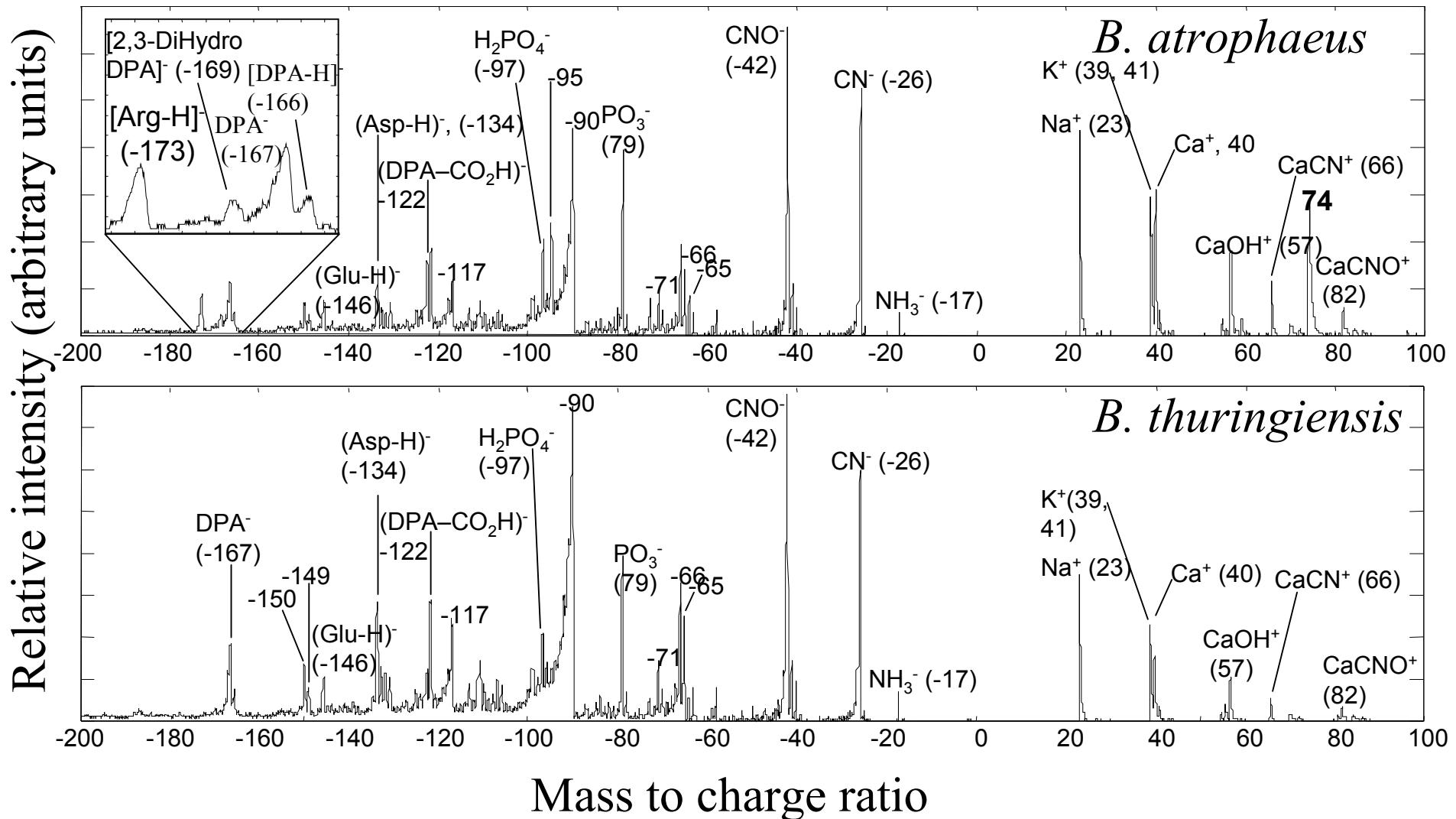
# *Bacillus* spores detected in a complex mixture

- BAMs is able to detect and identify *Bacillus* spores at the genus level from a complex background.
- No false positives.
- Low false negatives.
- Single spore detection
- Able to differentiate *Bacillus*, *Clostridia*, and fungal spores.
- Ability to identify unknown powder.

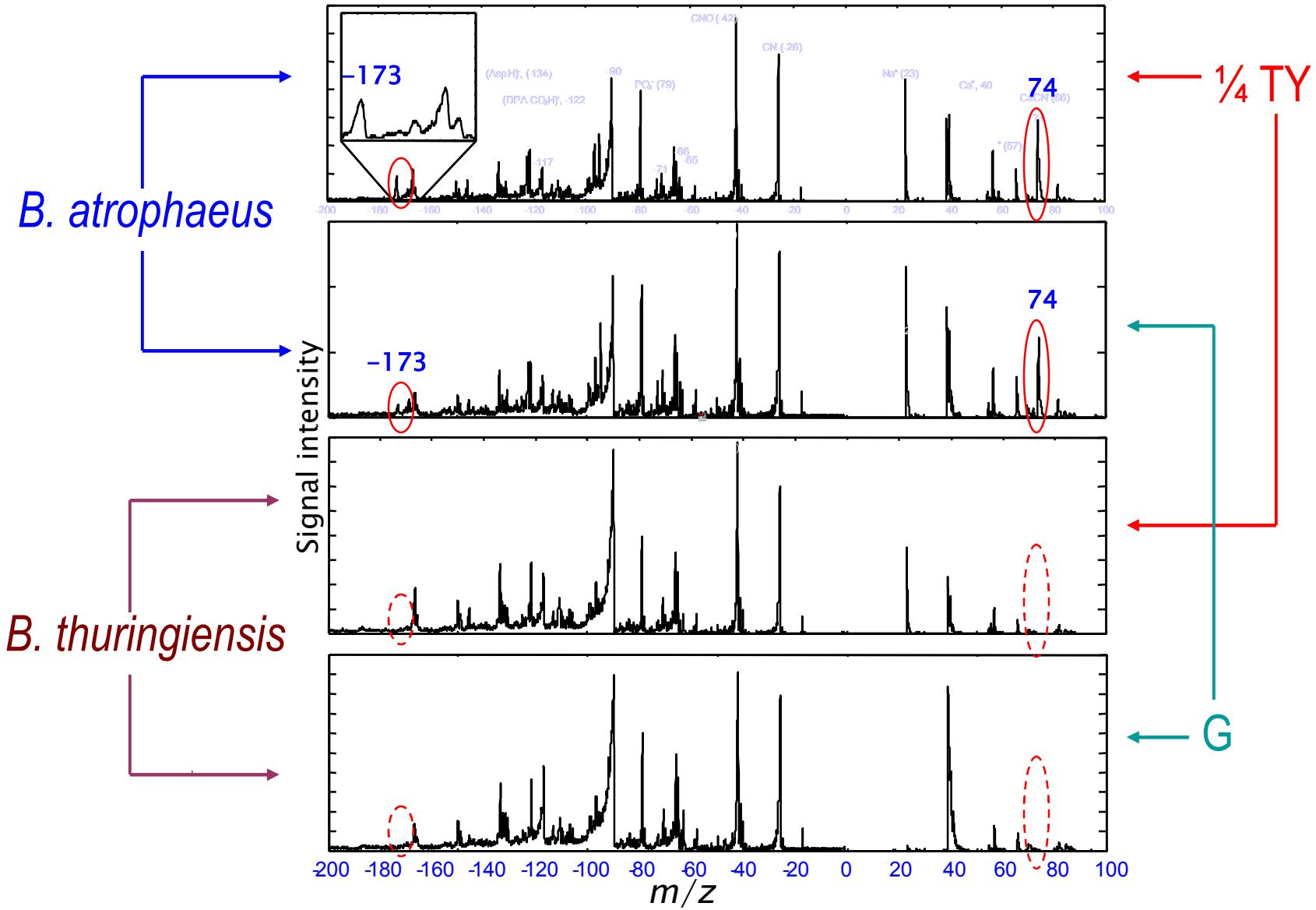
Mixed aerosol contains all components in legend *plus* Baking soda and powdered Sugar added to mix.



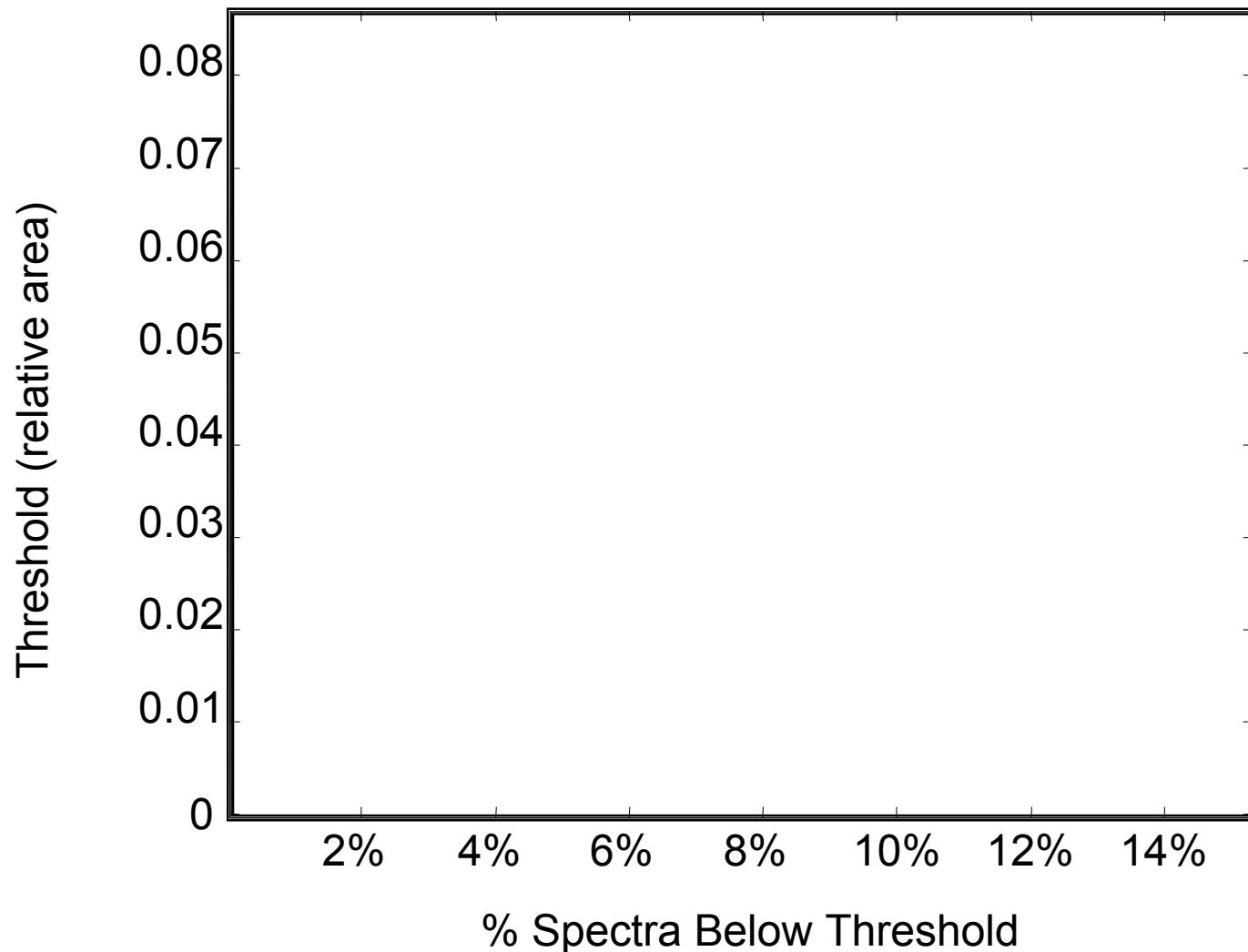
# Determining Spore Species (Blind)



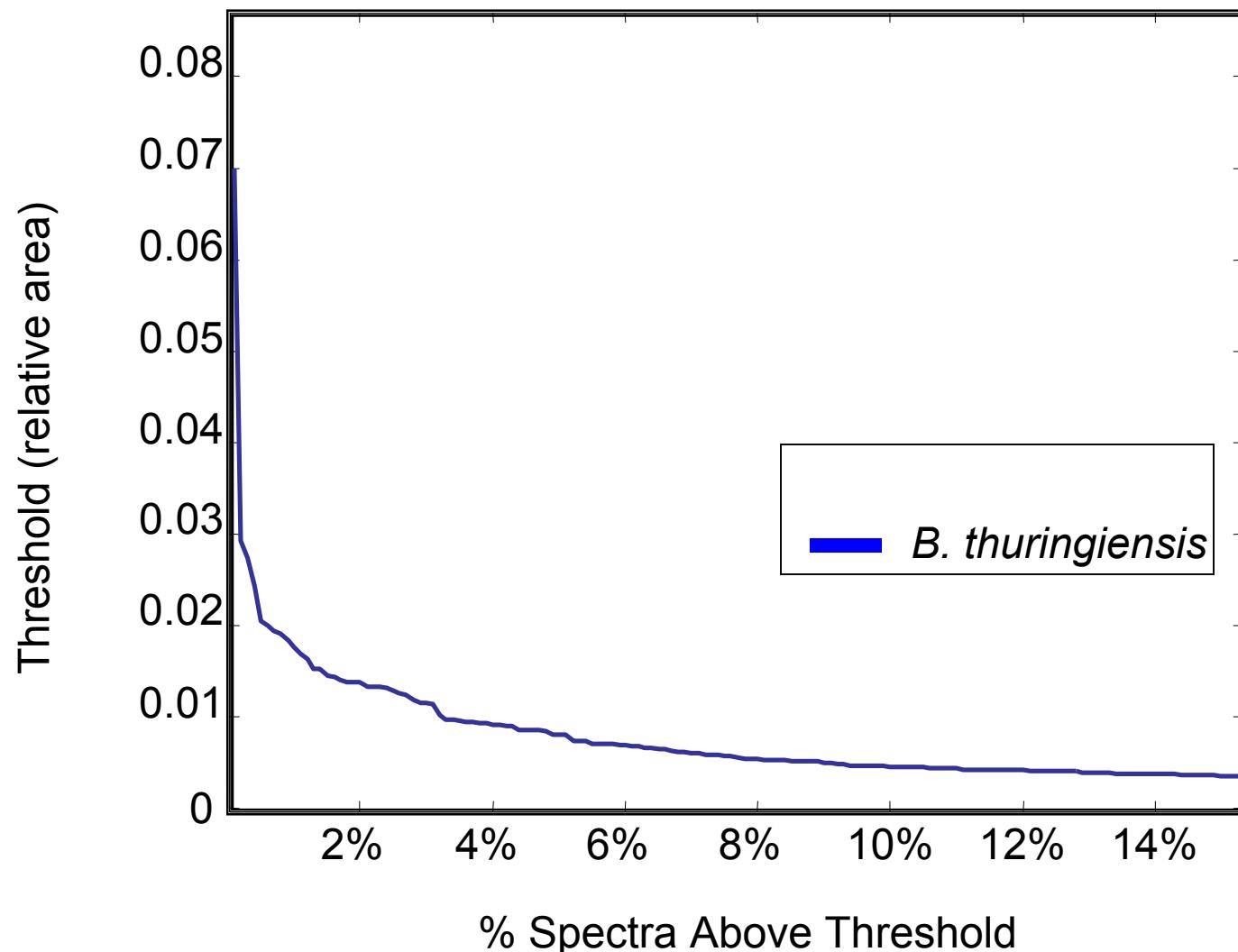
# *B. atrophaeus* and *B. thuringiensis*, two different media



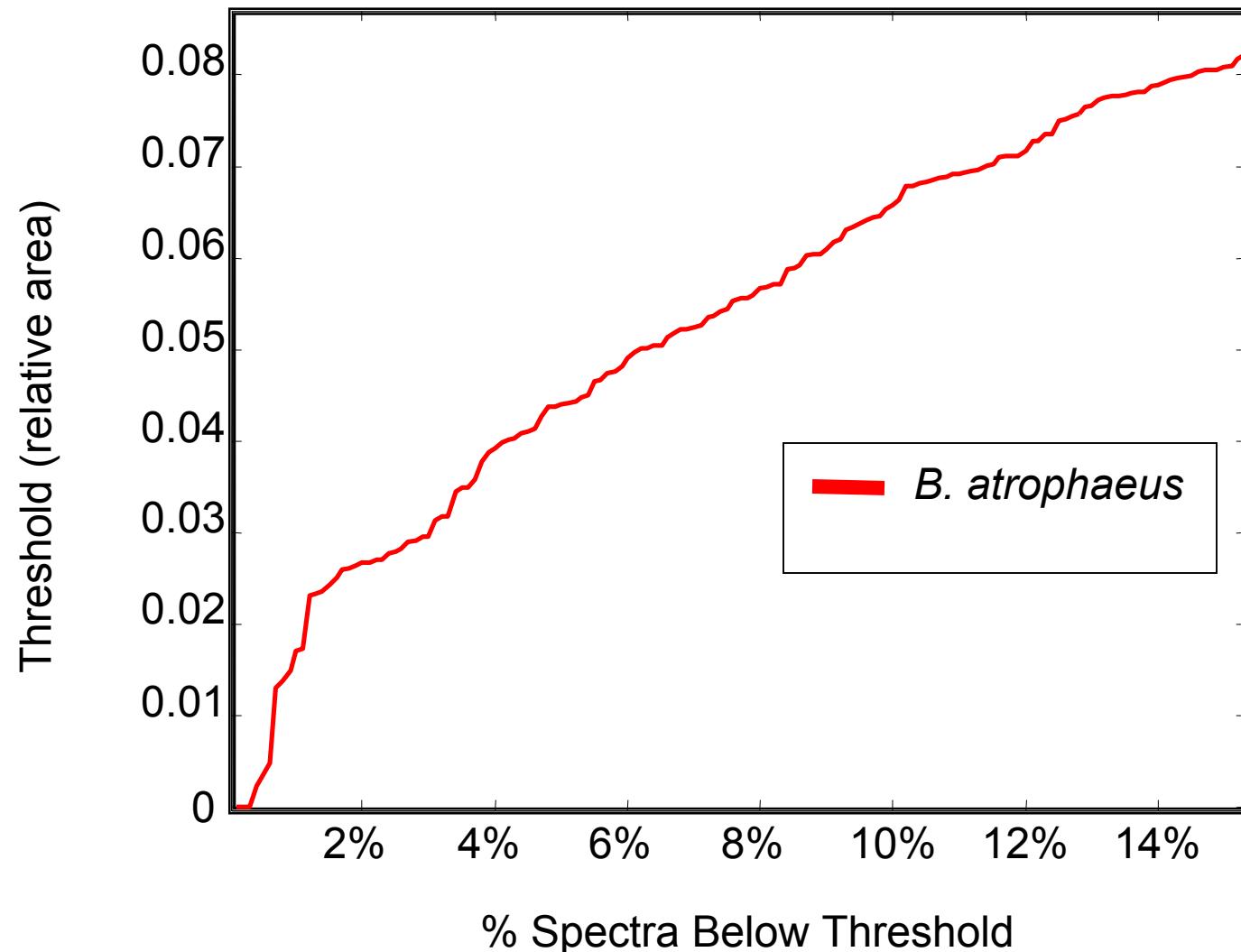
It is possible to plot the amount of a particular molecule in each sample



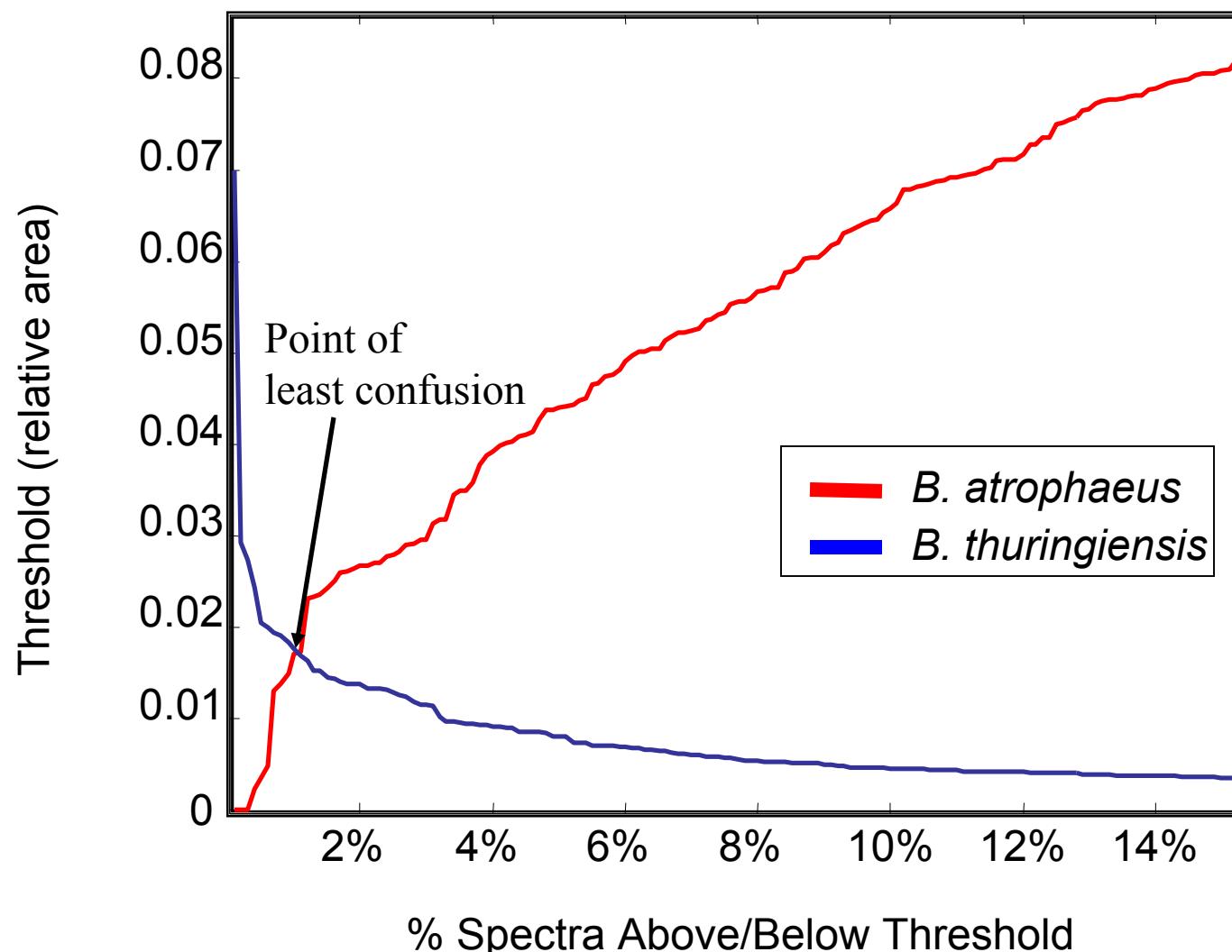
We can find out how many spectra have less than a certain amount...



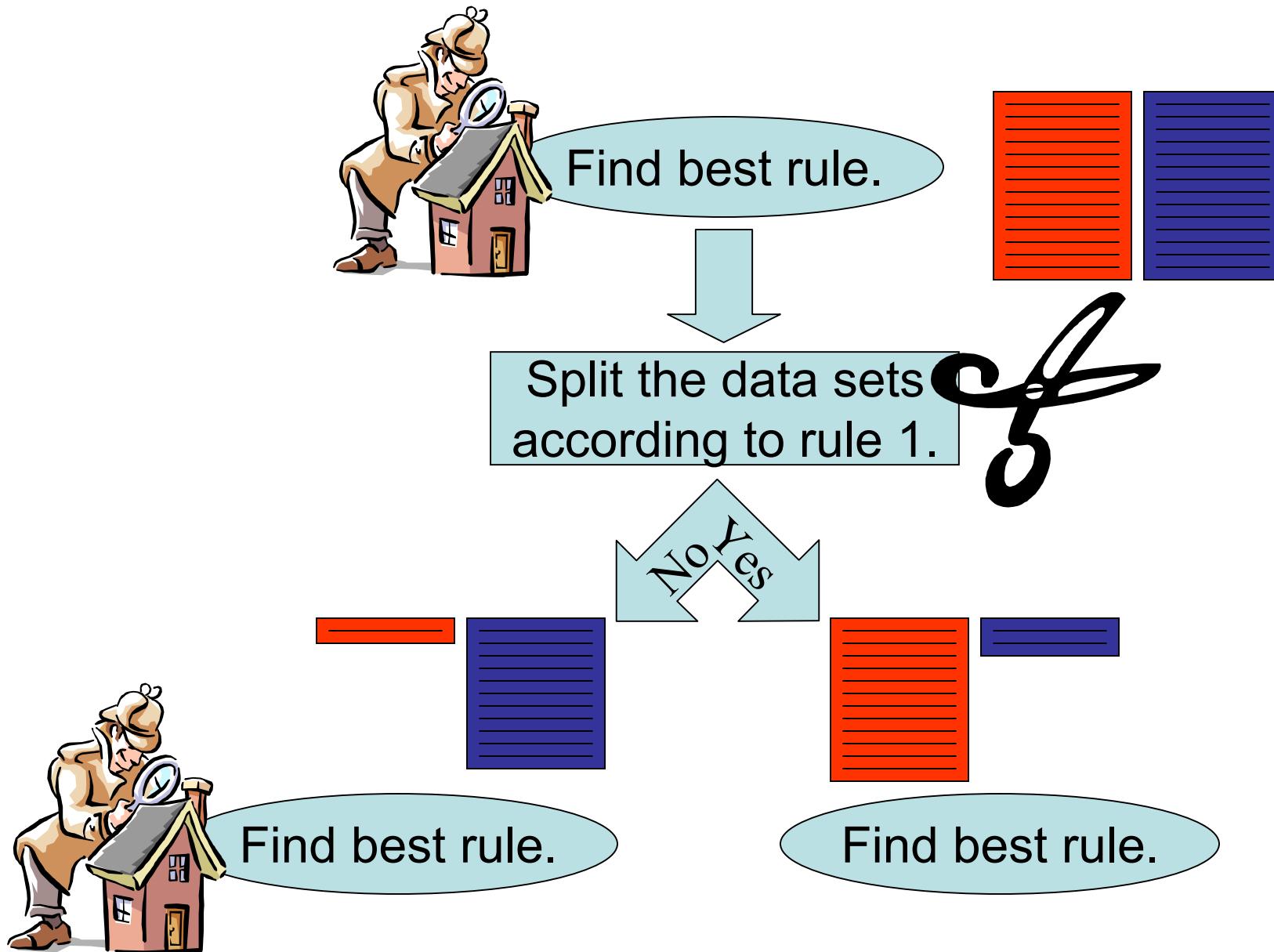
# Or more than a certain amount...



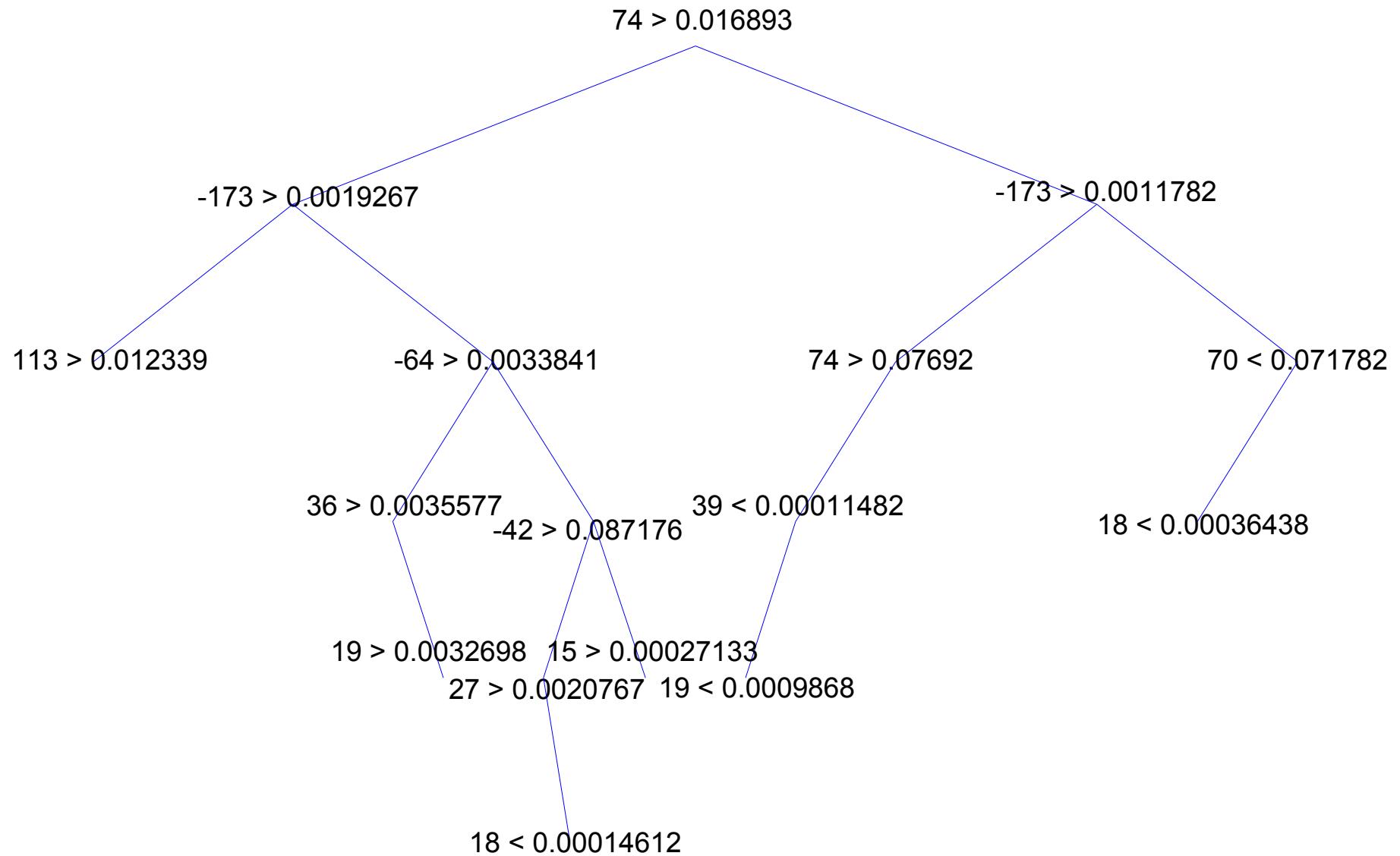
The point that the graphs cross is  
the optimal threshold.



# Rules-Tree Generation

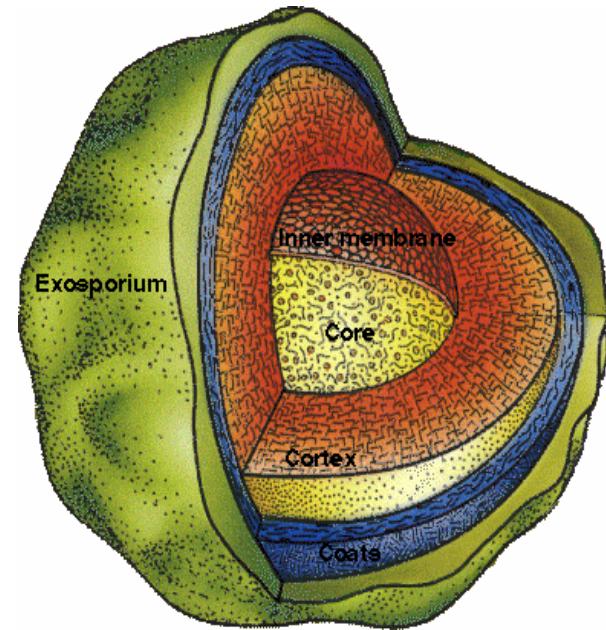


# *B. atrophaeus* vs. *B. thuringiensis*

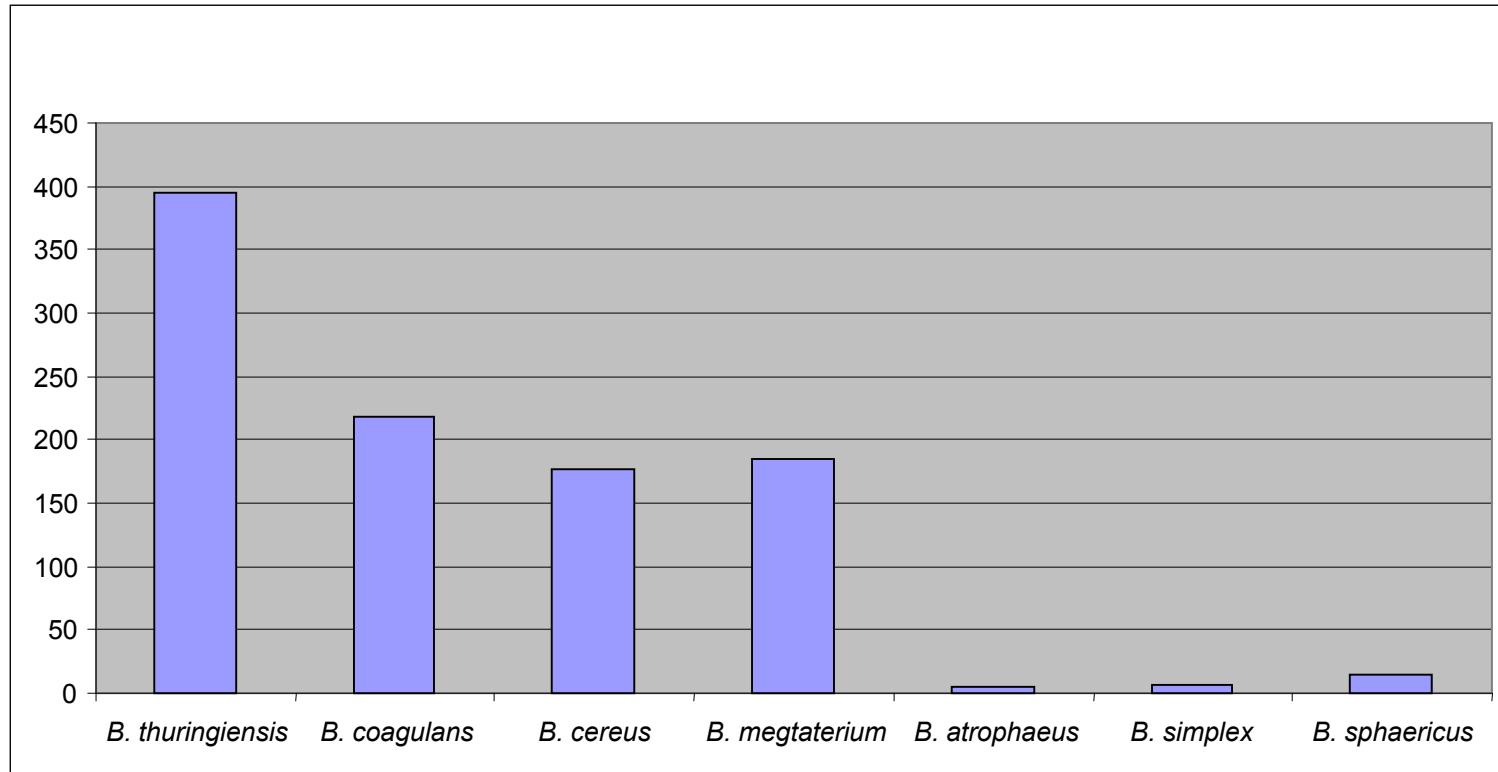


# *Bacillus* Spore Species Study

- 7 Species
  - *B. thuringiensis*
  - *B. coagulans*
  - *B. cereus*
  - *B. megaterium*
  - *B. atrophaeus*
  - *B. simplex*
  - *B. sphaericus*
- Run in duplicate, about 5K spectra each.
- 4 blind coded unknowns run as well.

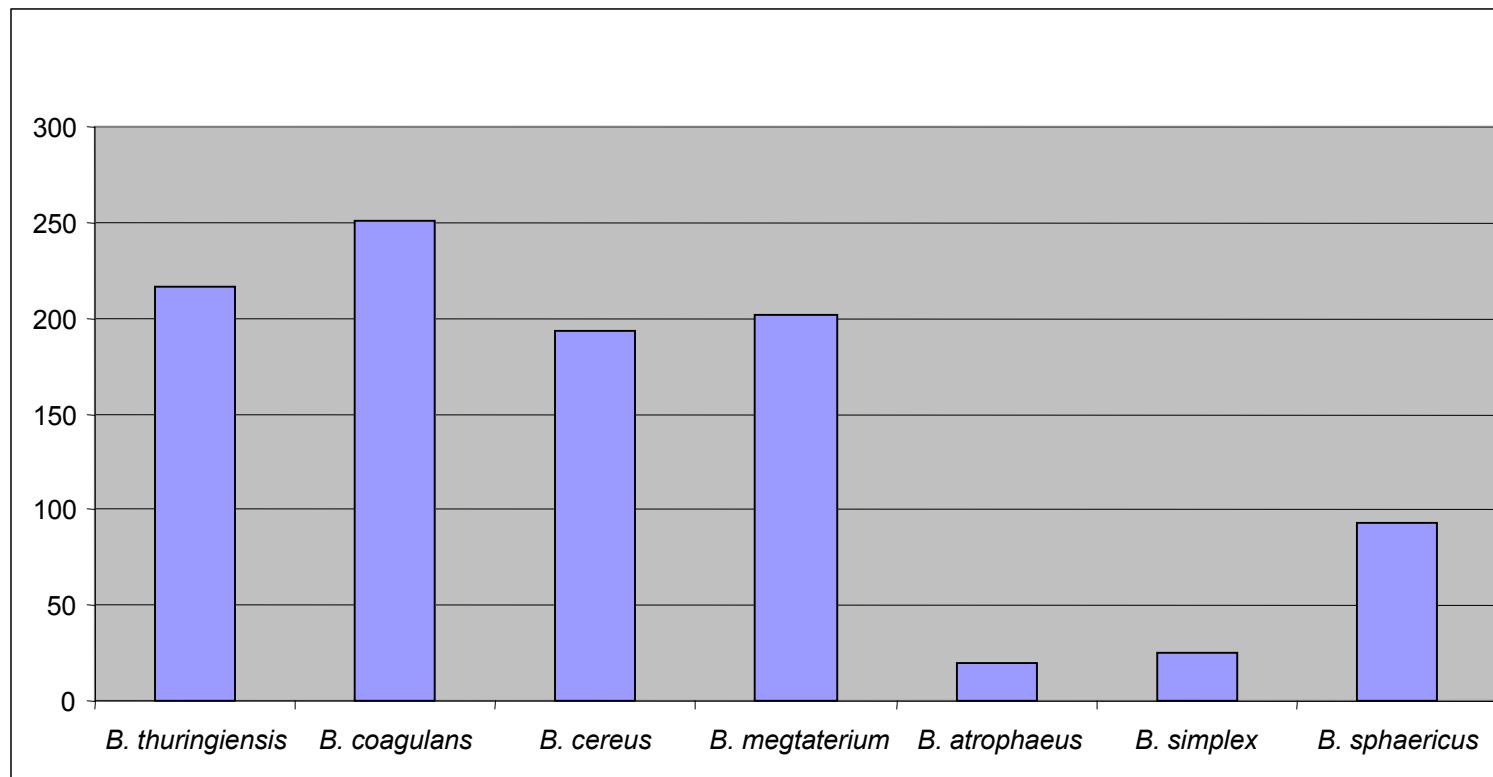


# *B. thuringiensis* Assignments



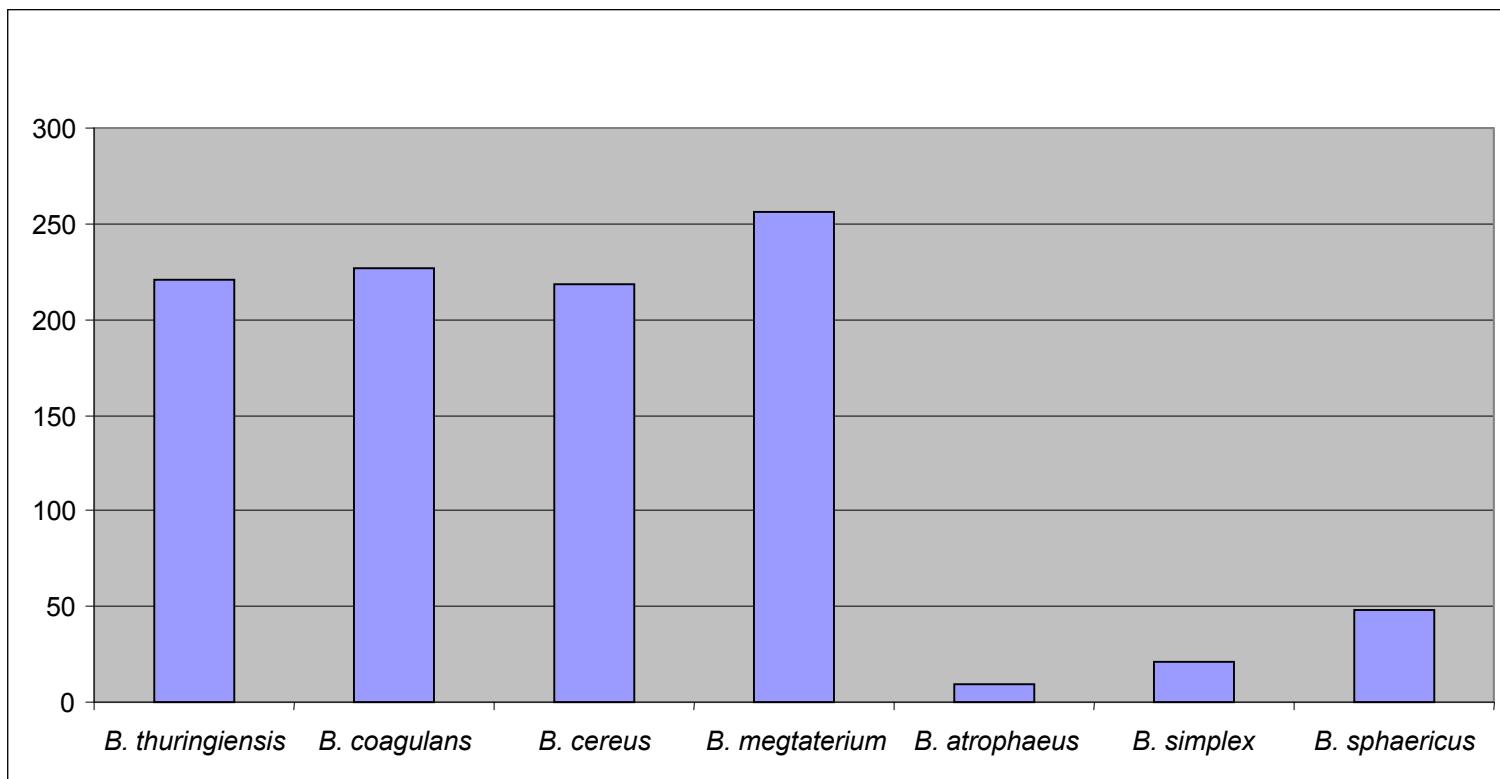
1000 Spectra

# *B. coagulans* Assignments



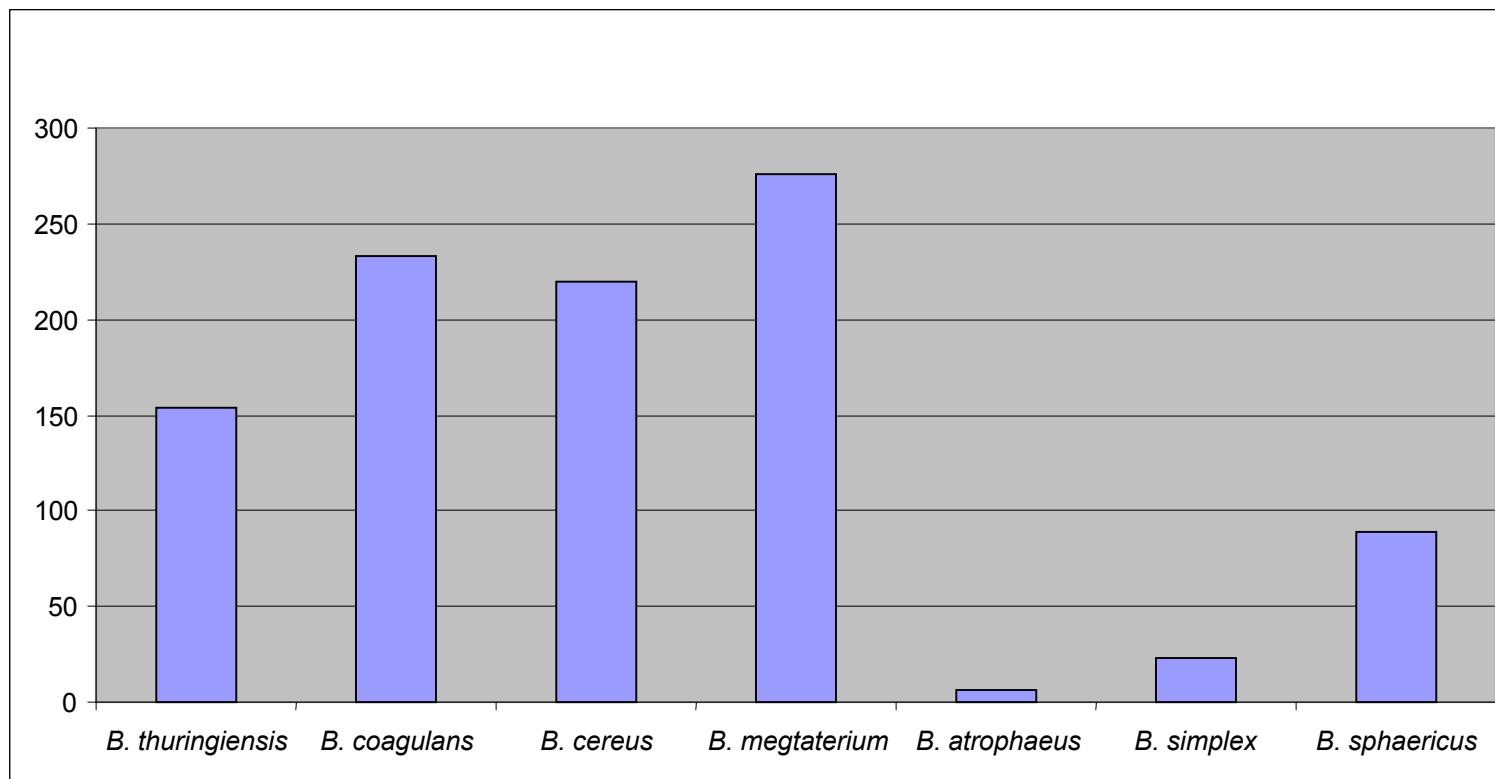
1000 Spectra

# *B. cereus* Assignments



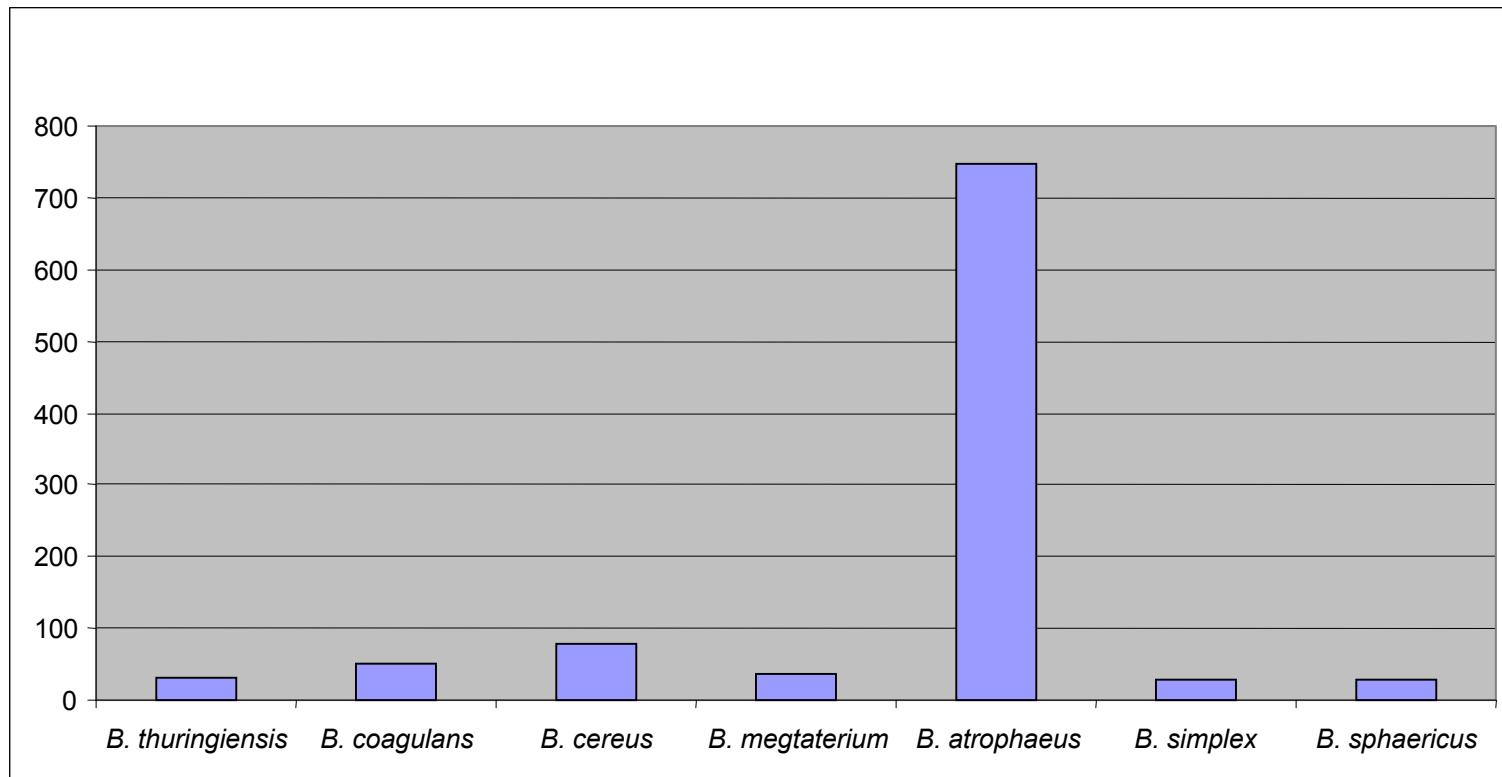
1000 Spectra

# *B. megaterium* Assignments



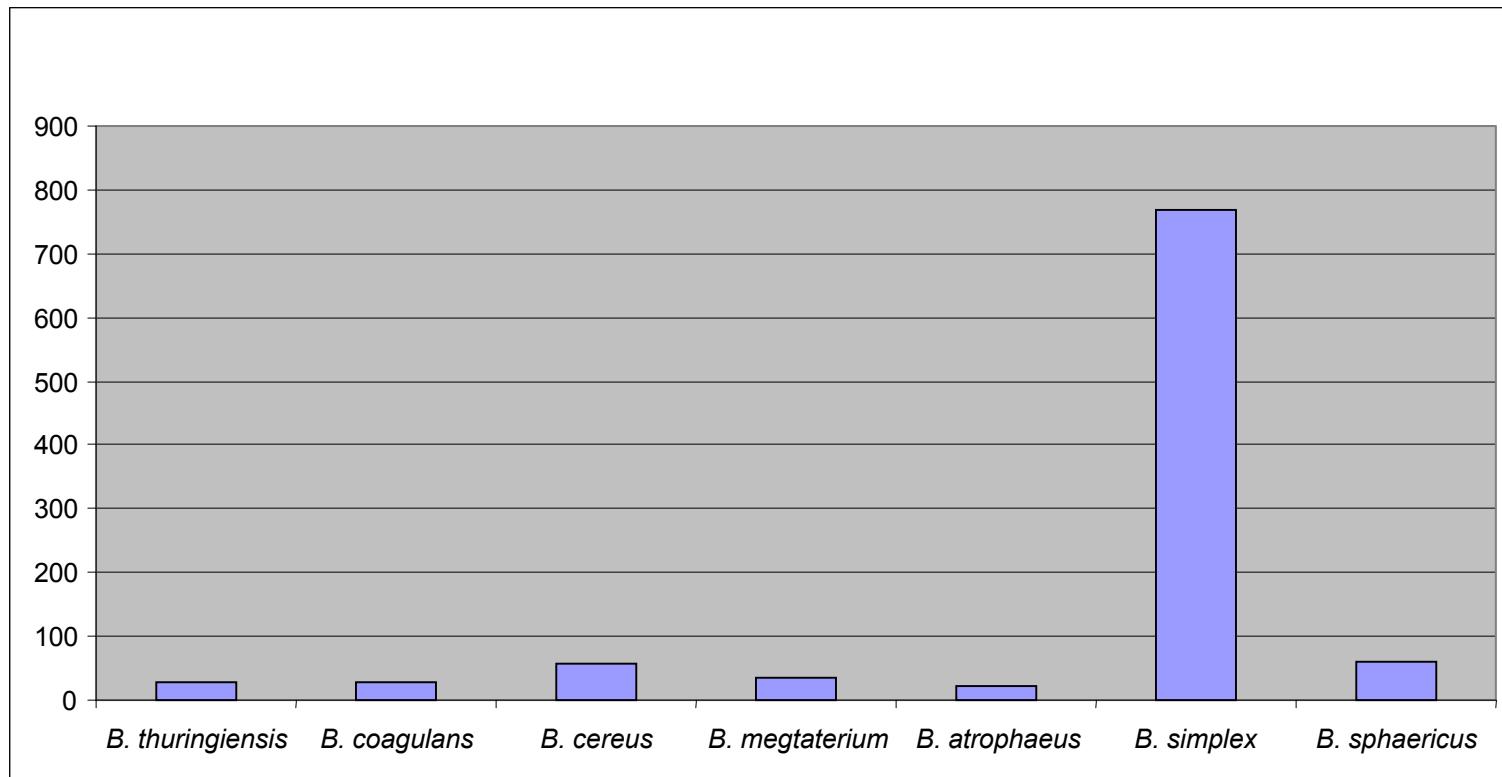
1000 Spectra

# *B. atrophaeus* Assignments



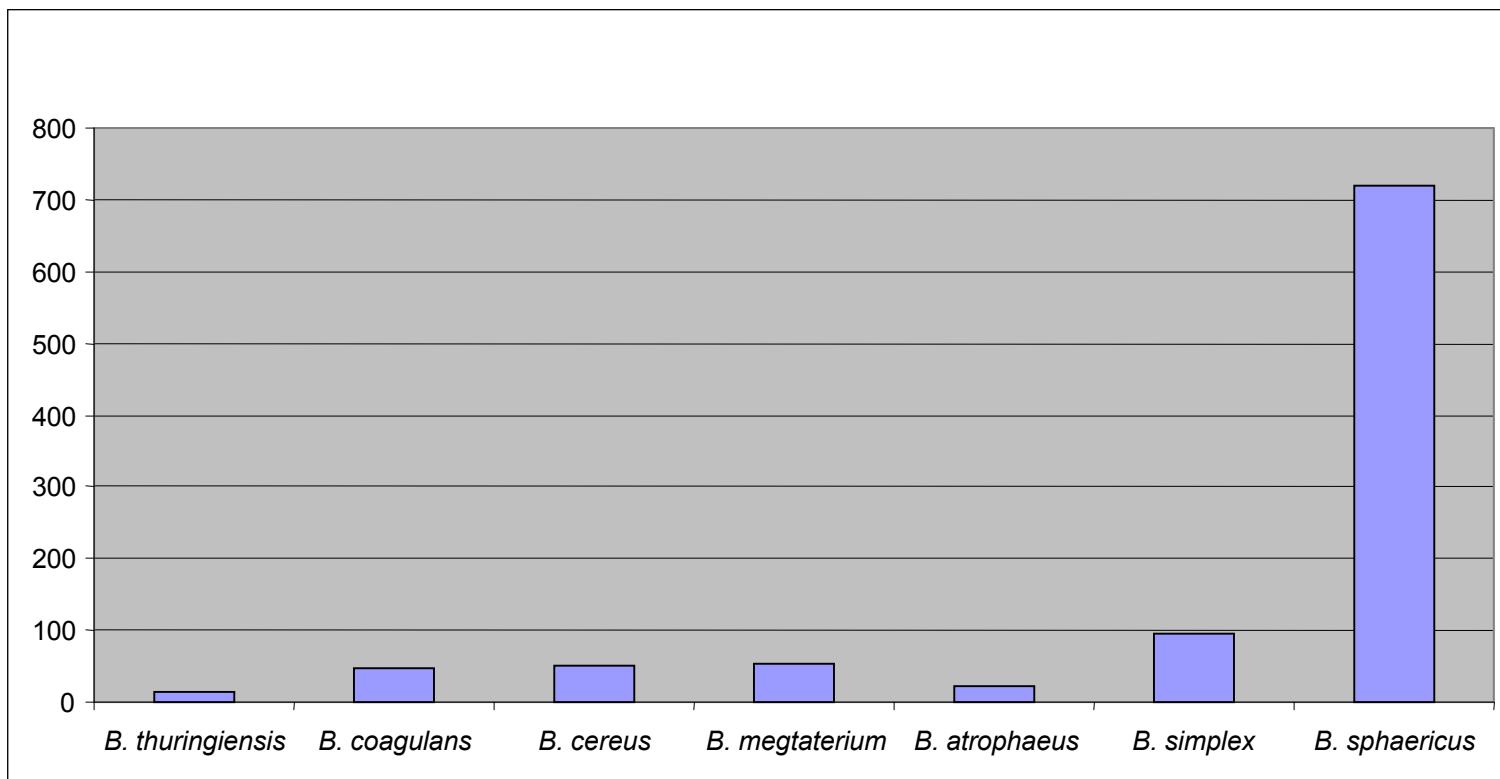
1000 Spectra

# *B. simplex* Assignments



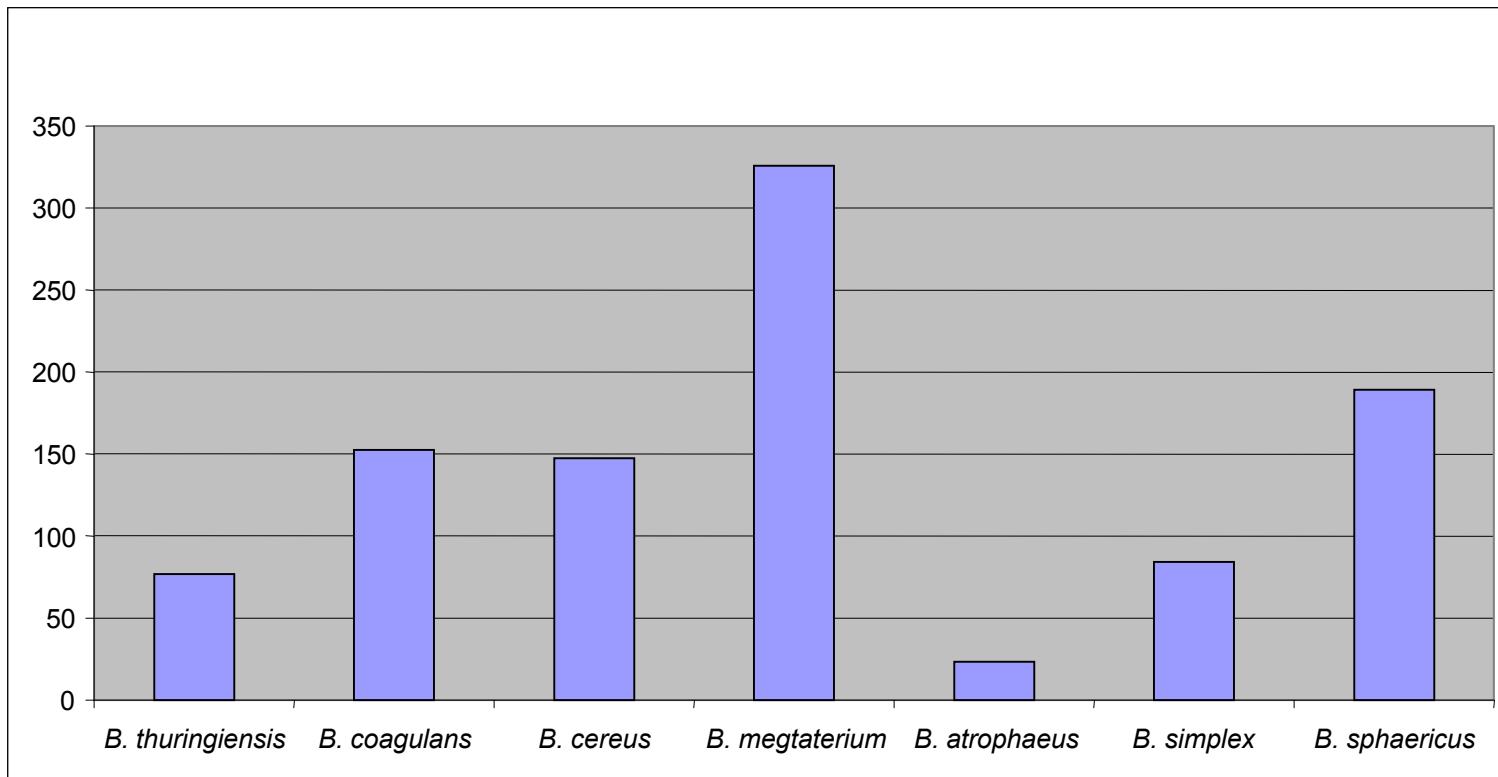
1000 Spectra

# *B. sphaericus* Assignments



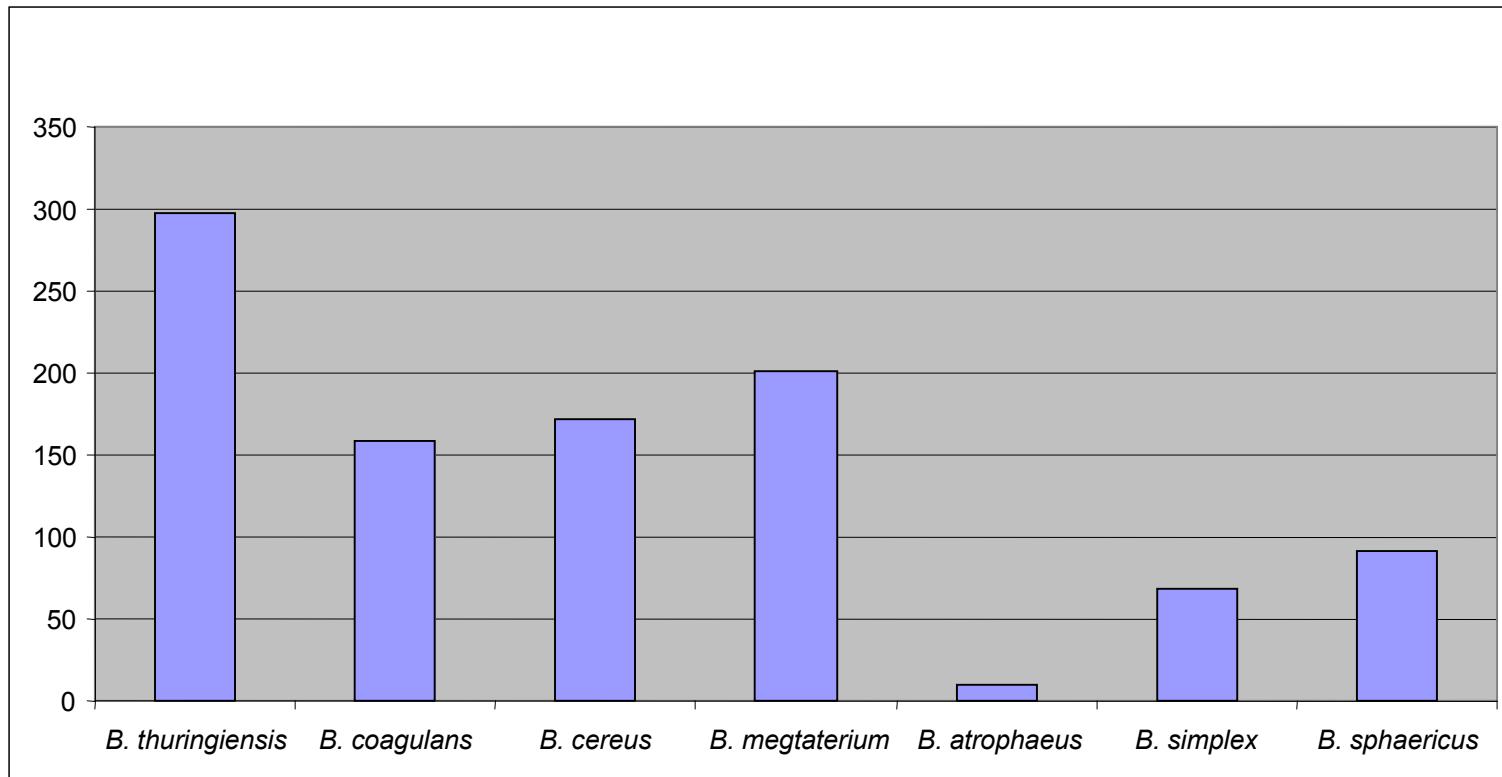
1000 Spectra

# Unknown 1 (*B. megaterium*)



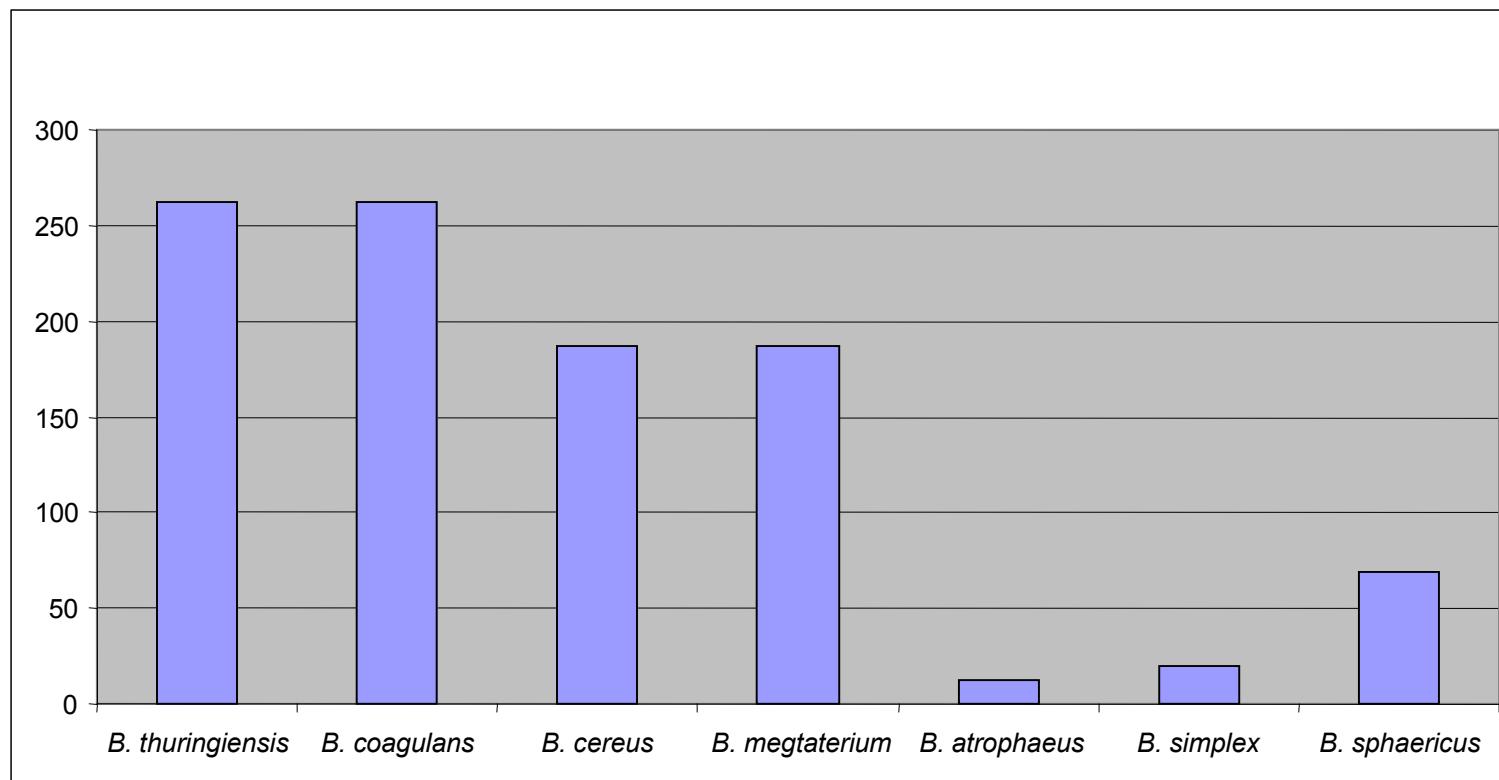
1000 Spectra

# Unknown 2( *B. thuringiensis*)



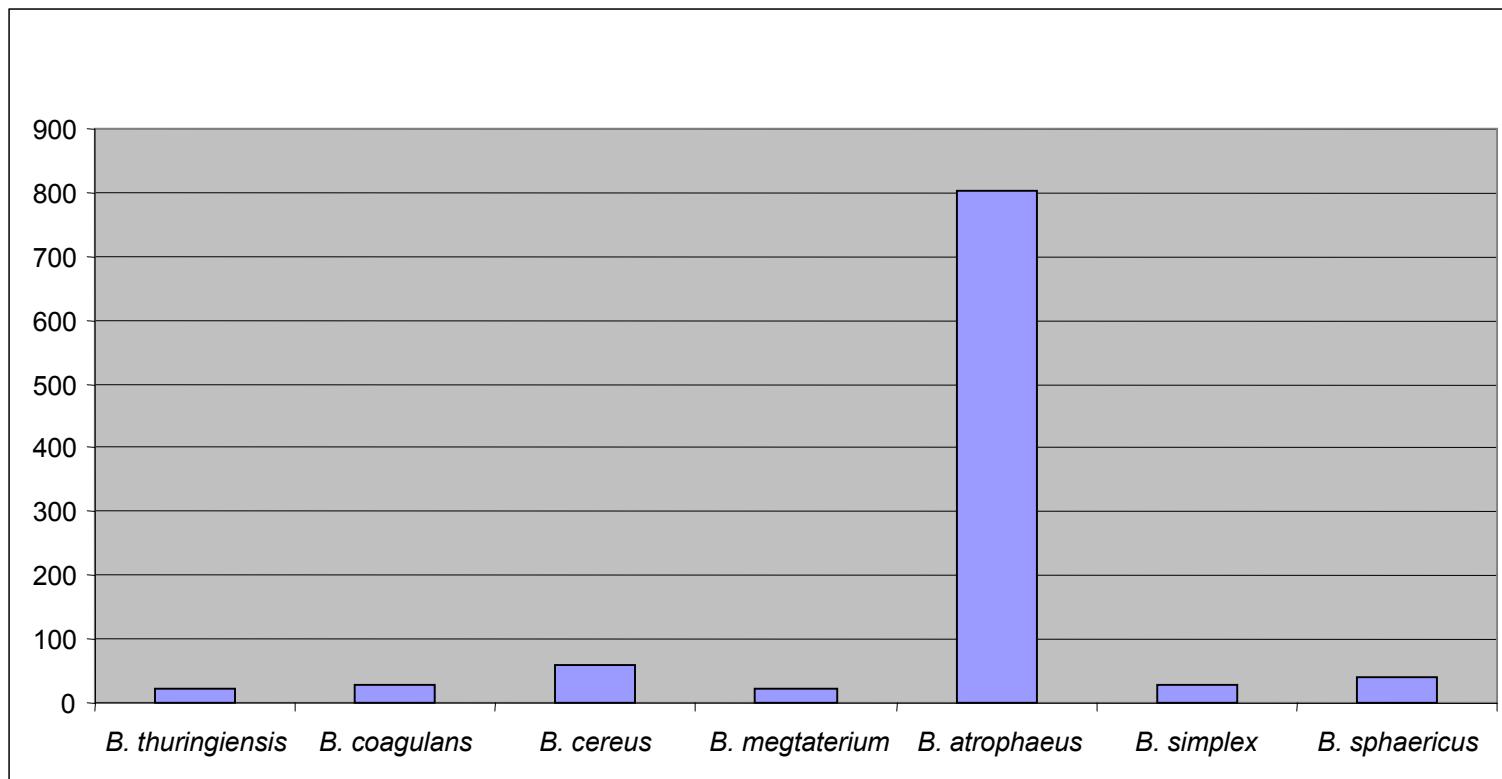
1000 Spectra

# Unknown 3 (*B. coagulans*)



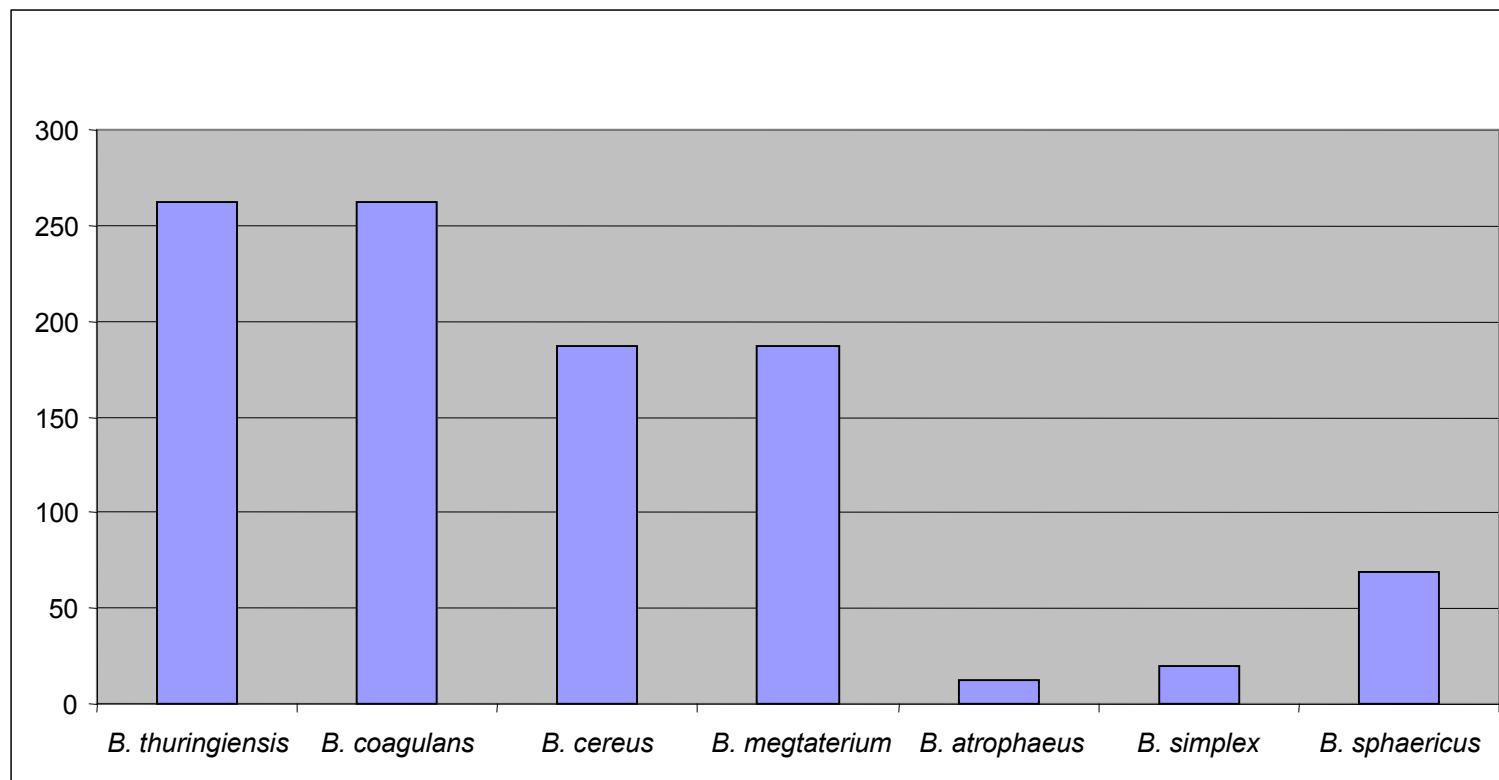
1000 Spectra

# Unknown 4 (*B. atrophaeus*)



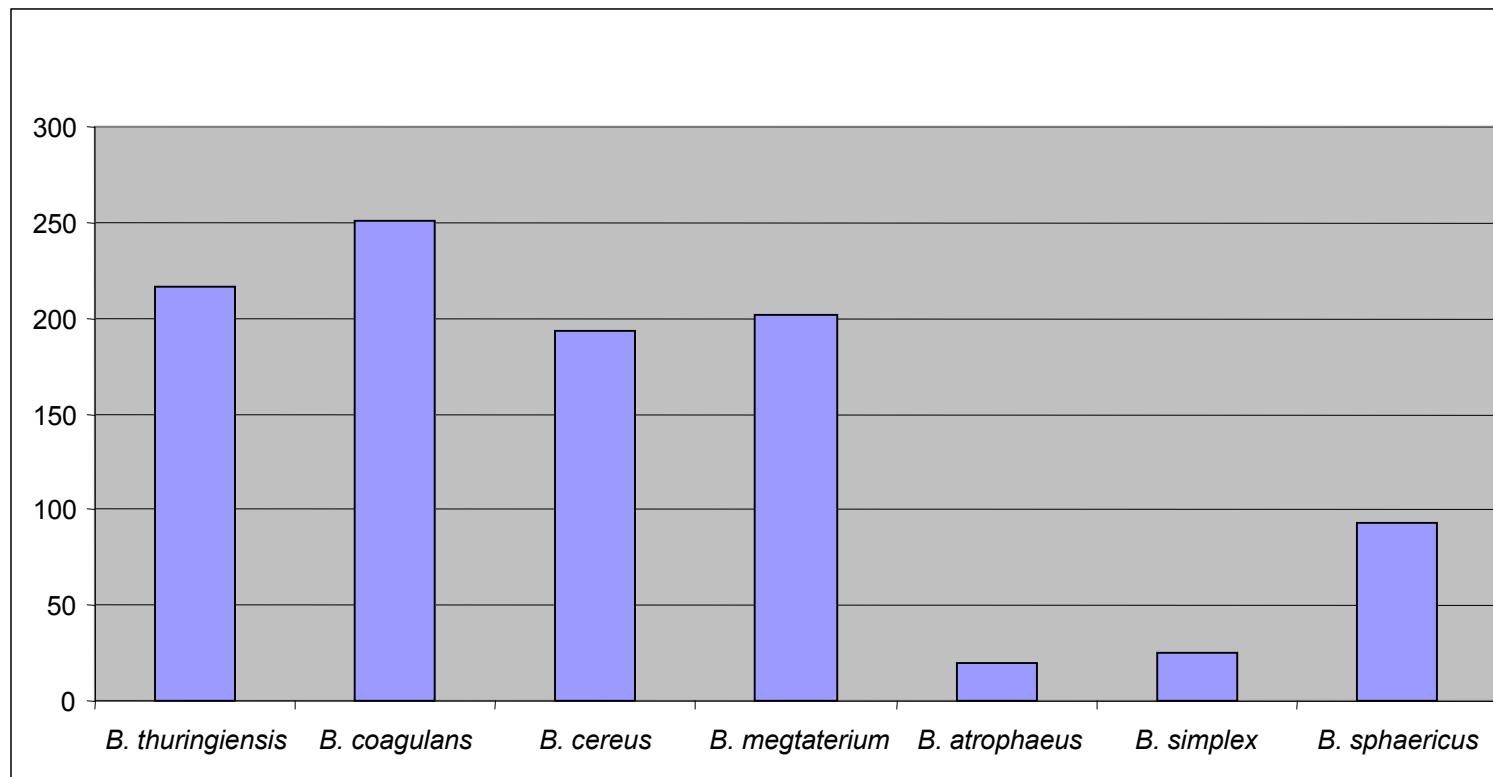
1000 Spectra

# Unknown 3 (*B. coagulans*)



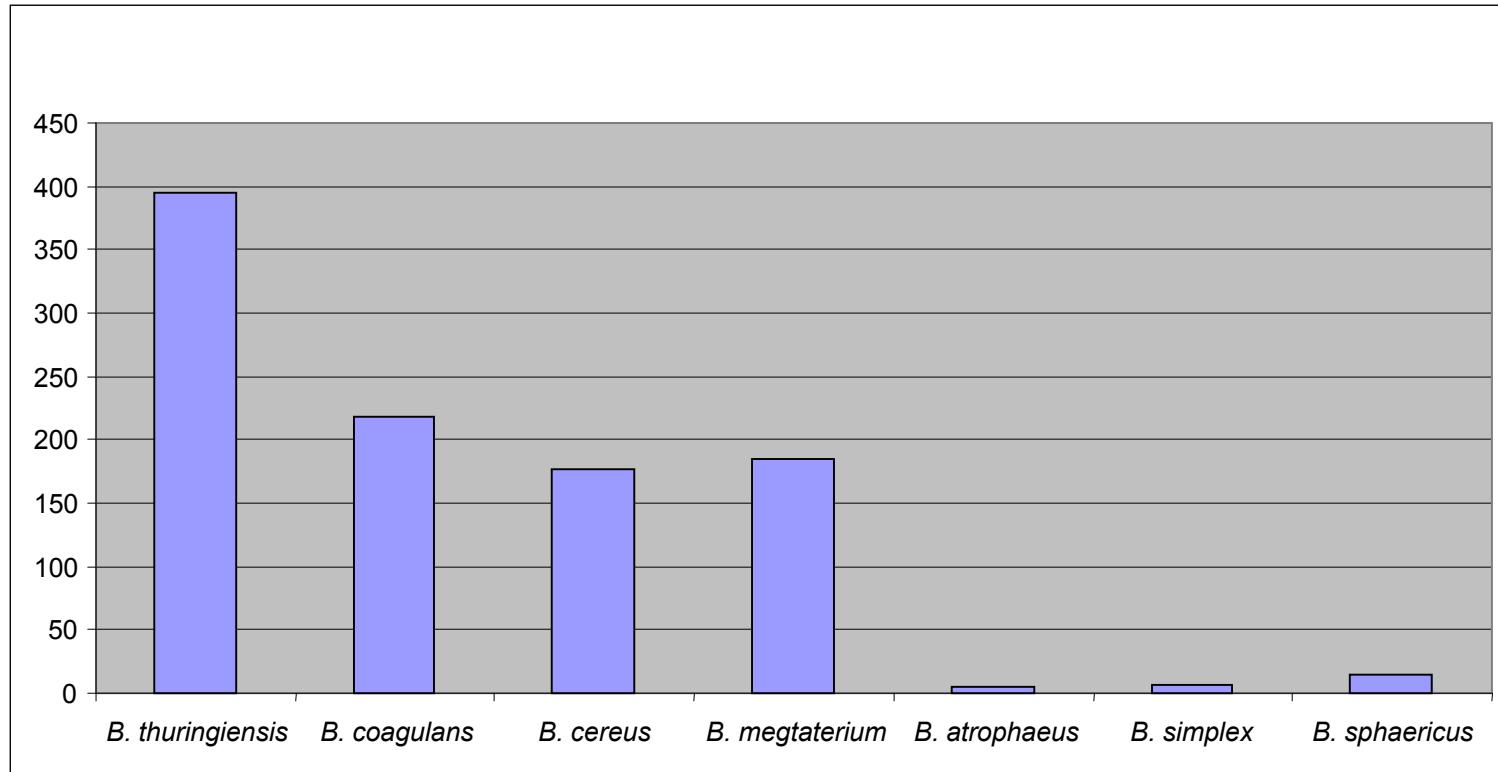
1000 Spectra

# *B. coagulans* Assignments



1000 Spectra

# *B. thuringiensis* Assignments



1000 Spectra

# Conclusions/Future Research

- BAMS is able to identify *Bacillus* spores from within a complex background
- BAMS can provide limited species information for microorganisms.
- Need to expand signature space
  - Proteins
  - Other metabolites
- Need to examine growth conditions impacts.

# Acknowledgements

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- \$LDRD

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